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# **Postmenopausal hot flushes, vascular health and hormone therapy**

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Academic dissertation

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# ABSTRACT

Vasomotor hot flushes are complained of by approximately three out of four postmenopausal women, but their frequency and severity show great individual variation. Estrogen withdrawal is a key factor of hot flushes, but the autonomic nervous system, regulating cardiovascular function, may also be involved. Hot flushes have been present in women attending observational studies, but they have been absent (or very mild) in randomized trials. Therefore, if hot flushes are a factor connected with vascular health, they could perhaps be one explanation for the divergence of cardiovascular data in observational versus randomized studies.

The present study was designed to determine the possible impact of vasomotor symptoms on vascular health. One hundred and fifty healthy, recently postmenopausal women showing a large variation in hot flushes were studied in regard to cardiovascular health by way of pulse wave analysis, ambulatory blood pressure and several biochemical vascular markers. In addition, the responses of these variables to hormone therapy were studied. The severity of hot flushes was assessed by using a two-week hot flush diary.

After the nitroglycerin challenge in pulse wave analysis, the time to the onset of the reflected wave (dependent on pulse wave velocity) was 9.2% shorter ( $p=0.014$ ), and the time to the first systolic peak (dependent on the rapid phase of ventricular ejection) was 12.3% shorter ( $p=0.025$ ) in asymptomatic women compared with women with severe hot flushes. This can be seen as evidence of vasodilatory reactivity associated with severe hot flushes, and thus, as a hot flush-related vascular benefit. Severe night-time hot flushes were accompanied by transient increases in ambulatory systolic ( $4.1\pm 10.5$  mmHg,  $p=0.061$ ) and diastolic ( $3.1\pm 6.8$  mmHg,  $p=0.032$ ) blood pressure and heart rate ( $3.0\pm 7.2$  beats/minute,  $p=0.043$ ), but the diurnal blood pressure profiles showed no significant difference between women without and with hot flushes of different severity. Vascular biomarkers were unaffected by hot flushes.

In the 6-month hormone therapy trial the women were classified as having either tolerable ( $\leq 3$  mild episodes/day, not likely to initiate hormone therapy in routine clinical practice) or intolerable ( $\geq 7$  moderate/severe episodes/day, likely to initiate hormone therapy) hot flushes. These groups were treated in a randomized order with transdermal estradiol hemihydrate gel (1 mg/day), oral estradiol valerate (2 mg/day) alone or in combination with medroxyprogesterone acetate (5 mg/day), or with placebo.

In women with tolerable hot flushes, oral estradiol led to decreases in the time to the first systolic peak (13.2%,  $p=0.028$ ) and the time to the reflected wave (8.4%,  $p=0.018$ ) after the nitroglycerin challenge. This potentially unfavorable vasoconstrictive effect was not seen in women with intolerable hot flushes or with the other treatment regimens. Additionally, 24-hour systolic ( $3.7\pm1.2$  mmHg,  $p=0.010$ ) and diastolic ( $1.8\pm0.8$  mmHg,  $p=0.003$ ) blood pressures rose in women with tolerable hot flushes receiving oral estradiol, whereas decreases ( $-1.2\pm1.2$  mmHg and  $-2.1\pm0.8$  mmHg, respectively) were detected in women with intolerable hot flushes. Daytime systolic and diastolic blood pressures also increased ( $3.0\pm1.3$  mmHg,  $p=0.017$  and  $1.8\pm0.9$  mmHg,  $p=0.003$ , respectively) in women with tolerable hot flushes with oral estradiol treatment, but the same therapy led to falls ( $-1.9\pm1.3$  mmHg and  $2.4\pm0.9$  mmHg, respectively) in women with intolerable hot flushes. No such effects were observed as regards transdermal estradiol or oral estradiol accompanied by medroxyprogesterone acetate. The responses of lipids, lipoproteins, C-reactive protein and sex hormone-binding globulin to hormone therapy showed no association with hot flush status.

In conclusion, hot flushes may be associated with an increased vasodilatory, and thus a beneficial vascular status. Furthermore, oral estradiol leads to vasoconstrictive changes, and thus to possible vascular harm, but only in women whose hot flushes are so mild that they would probably not lead to the initiation of hormone therapy in clinical practice. Such potential vascular harm of hormone therapy was not seen in women with intolerable hot flushes, or with the other treatment regimens.

Taken as a whole, hot flush status contributes to cardiovascular health before and during hormone therapy. Severe hot flushes associate with enhanced vascular reactivity. Potentially unfavorable vascular effects of oral hormone therapy are seen mainly in women without troublesome hot flushes. Thus, if estrogen is prescribed for indications other than for the control of hot flushes, transdermal route of administration should be favored.

# LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

I Evidence for a role of hot flushes in vascular function in recently postmenopausal women. Tuomikoski P, Ebert P, Haapalahti P, Hautamäki H, Rönnback M, Ylikorkala O, Mikkola TS. *Obstetrics and Gynecology* 2009;113:902–8.

II Vasomotor hot flushes and 24-hour ambulatory blood pressure in recently postmenopausal women. Tuomikoski P, Haapalahti P, Ylikorkala O, Mikkola TS. *Annals of Medicine*: in press.

III Biochemical markers for cardiovascular disease in recently postmenopausal women with or without hot flushes. Tuomikoski P, Mikkola TS, Härmäläinen E, Tikkanen MJ, Turpeinen U, Ylikorkala O. *Menopause* 2010;17:145–51.

IV Effect of hot flushes on vascular function: A randomized, controlled trial. Tuomikoski P, Ebert P, Groop P-H, Haapalahti P, Hautamäki H, Rönnback M, Ylikorkala O, Mikkola TS. *Obstetrics and Gynecology* 2009;114:777–85.

V Impact of hot flushes on 24-hour ambulatory blood pressure in normotensive women: A placebo-controlled trial on postmenopausal hormone therapy. Tuomikoski P, Haapalahti P, Sarna S, Ylikorkala O, Mikkola TS. *Annals of Medicine*: in press.

VI Hot flushes and biochemical markers for cardiovascular disease: A randomized trial on hormone therapy. Tuomikoski P, Mikkola TS, Tikkanen MJ, Ylikorkala O. *Climacteric*: in press.

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## ABBREVIATIONS

AIx	Augmentation index
apoA-I	Apolipoprotein A-I
apoB	Apolipoprotein B
BMI	Body mass index
BP	Blood pressure
CEE	Conjugated equine estrogens
CHD	Coronary heart disease
CI	Confidence interval
CVD	Cardiovascular disease
EFI	Endothelial function index
EPT	Estrogen-progestagen therapy
ET	Estrogen-only therapy
FEI	Free estradiol index
FMD	Flow-mediated dilation
FSH	Follicle-stimulating hormone
HDL	High-density lipoprotein cholesterol
HERS	Heart and Estrogen/progestin Replacement Study
HFWS	Hot flush weekly weighted score
HR	Hazard ratio
hs-CRP	High-sensitivity C-reactive protein
HT	Hormone therapy
LDL	Low-density lipoprotein cholesterol
LH	Luteinizing hormone
Lp (a)	Lipoprotein (a)
MPA	Medroxyprogesterone acetate
OR	Odds ratio
PWA	Pulse wave analysis
SD	Standard deviation
SEM	Standard error of mean
SHBG	Sex hormone-binding globulin
RR	Risk ratio
VLDL	Very low-density lipoprotein cholesterol
WHI	Women's Health Initiative

# INTRODUCTION

Hormone therapy (HT) has long been used for the treatment of menopausal symptoms, such as hot flushes. Results from an abundance of observational studies showed a 30–50% reduction in the risk of cardiovascular disease (CVD) in current users of HT. Thus, over a decade ago HT was commonly recommended for the prevention of CVD (*Mikkola and Clarkson 2002, Mikkola and Ylikorkala 2005*). However, results from randomized, placebo-controlled trials, such as the Heart and Estrogen/progestin Replacement Study (HERS) (*Hulley et al. 1998, Grady et al. 2002*) and the Women’s Health Initiative (WHI) (*Rossouw et al. 2002, Manson et al. 2003*) indicated an increased risk of CVD in HT users (*Hodis and Mack 2008*). These findings, widely debated in both the medical and lay press, led to major changes in clinical practice when millions of women around the world discontinued use of HT (*Utian 2007*).

Numerous plausible explanations for these divergent data have been suggested, including the “healthy woman” bias in observational studies, and possible differences in treatment regimens and study populations between observational and randomized studies (*Mikkola and Clarkson 2002, Grodstein et al. 2003*). The presence of vasomotor hot flushes has also emerged as one possible explanation (*Mikkola and Ylikorkala 2005, van der Schoouw and Grobbee 2005*). Hypoestrogenism certainly contributes to the onset of hot flushes, but the autonomic nervous system, which regulates the vascular bed, among other things, may be involved (*Freedman 1998*). In clinical practice women with severe hot flushes seek HT treatment, which is the most effective treatment against for them, and thus, such women have been included in HT groups in observational studies. In contrast, hot flushes have been absent, or very mild, in women taking part in randomized clinical trials, where women with severe hot flushes have been excluded from participation.

Differences also exist in treatment regimens, e.g. in United States the use of conjugated equine estrogens (CEE) is favored, whereas in Europe estradiol-based regimens dominate (*Mikkola and Clarkson 2002, Grodstein et al. 2003, van der Schoouw and Grobbee 2005*). It is also possible that progestagens possess direct or indirect vascular effects (*Kob and Sakuma 2004*). In this regard medroxyprogesterone acetate (MPA) has been most widely discussed, because MPA as a continuous complement of CEE was present in both the HERS and WHI trials. However, it is still unsettled whether the vascular effect of MPA is beneficial or harmful (*Clarkson and Appt 2003*).

Moreover, oral and transdermal administration of estrogen leads to different estrogenic milieus (*Kuhl 2005*) and thus, the cardiovascular effects of orally and transdermally administered HT may differ (*Godsland 2001*).

The present studies explored the potential impact of hot flushes of different severity on various factors reflecting vascular health before and during six months of oral or transdermal estradiol, the former with and without MPA.

# REVIEW OF THE LITERATURE

## Menopause

In 2008 in Finland 191 000 women were between 50–54 years of age (*Statistics Finland 2009*), when women typically enter natural menopause (*Greendale 1999*). Menopause is a consequence of the slow deterioration of ovarian function. The primary trigger for ovarian senescence is not known, but genetically controlled apoptosis of the ovarian cells is involved (*Vaskivuo and Tapanainen 2003*). Therefore, it is no surprise that menopausal age shows a significant familial tendency (*de Bruin et al. 2001, Murabito et al. 2005*). Menopause is defined as the onset of the last menstruation, followed by 6–12 months of amenorrhea and therefore, this diagnosis can only be made retrospectively. Menopause before the age of 40 is defined as premature, and it is considered a disease-like condition. Some epidemiological factors, such as smoking, low body mass index (BMI), menstrual irregularities and nulliparity are associated with earlier menopause (*Barton et al. 2001, Nelson 2008*), whereas data on the influence of age at menarche and on oral contraceptive use are controversial (*Harlow and Signorello 2000*). Furthermore, socioeconomic factors, such as a low degree of education, unemployment or being divorced or widowed are independent risk factors of early menopause. Race and ethnicity are also associated with the timing of natural menopause (*Gold et al. 2001*), and for instance, Latin women experience menopause approximately nine months earlier than non-Latin white women, while Japanese race/ethnicity is associated with later menopause (*Henderson et al. 2008*). Furthermore, hysterectomy may be associated with 1–2 years earlier onset of menopause (*Lobo 2007*). It is also noteworthy that hypercholesterolemia and hypertension are predisposing factors as regards both earlier menopause and CVD (*Kok et al. 2006*).

During the menopausal transition the level of inhibin B originating from the ovaries decreases and the levels of pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) increase (Figure 1). As the level of FSH rises, numerous small ovarian follicles undergo activation, and this leads to increases in circulating estradiol levels at an early stage of menopausal transition. However, this is only a transient phenomenon and is not seen in all women, and hypoestrogenism is the ultimate outcome of menopause.

With increasing age the levels of FSH and LH continue to rise, but despite this, follicle depletion progresses (*Burger et al. 2007, Kase 2009*). Estradiol produced by the ovaries is the main estrogen during the fertile years, whereas after menopause circulating levels of estradiol are low (usually  $<20$  pmol/L), and estrone (usually  $<300$  pmol/L), produced by aromatization in fatty tissue, is the dominating estrogen after menopause. After menopause circulating levels of FSH and LH continue to rise for several years, but in later menopause these levels go down. The levels of LH are of importance because they stimulate androgen secretion from the ovarian stroma. The ovaries are not the only source of androgens, and they are also derived from estrogen aromatization in fatty tissue (*Kase 2009*).

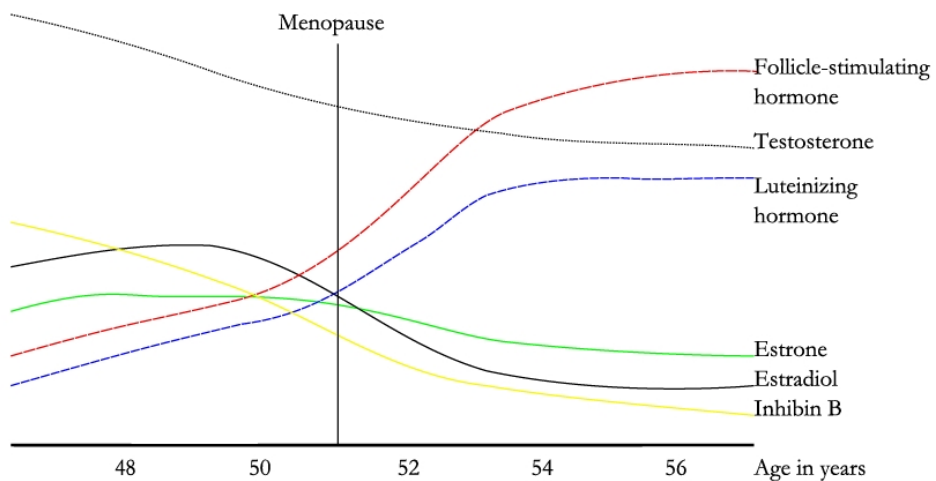


Figure 1. Schematic presentation of the changes in some reproductive hormones and inhibin B during perimenopause (*modified from Strauss and Barbieri 2004*).

## Hot flushes

The most typical features of menopause are hot flushes, also referred to as hot flashes or vasomotor symptoms, which are present in up to 75–80% of all women in menopausal age groups (*Dennerstein et al. 2000, Stearns et al. 2002, Deecher and Dorries 2007*). The frequency of hot flushes usually peaks within the year of the last menstruation (*Nelson 2008, Santoro 2008*). In most women hot flushes subside with advancing age, but approximately 20% of women continue to have hot flushes several years after menopause, and in many women symptoms may persist well into their seventies (*Santoro 2008*).

Hot flushes are characterized by rapid episodes of flushing/reddening of the skin and sensations of heat and sweating. They usually start from the chest and rise up to the neck and face, and hot flushes may sometimes be followed by chills (*Deecher and Dorries 2007, Sturdee 2008*). Hot flushes are often accompanied by palpitations, anxiousness and broken sleep. There is great individual variation in the frequency and severity of flushes, ranging from sensations lasting from a few seconds to up to an hour, and reoccurring only seldom or almost instantaneously (*Nelson 2008*). Approximately 20% of women regard hot flushes as intolerable (*Hickey et al. 2005, Skurnick et al. 2009*), but in almost all women hot flushes may to some extent endanger adequate sleep and lead to a number of other symptoms, such as nervousness or depressed mood, which are more or less secondary to hot flushes. As a net consequence of hot flushes the overall quality of life significantly decreases (*Polo-Kantola et al. 2001, Utian 2005, Rapkin 2007, Ensrud et al. 2009, Luoto 2009*).

The etiology of hot flushes is not known. They seem to be preceded by a rise in core body temperature (*Freedman 1998*) (Figure 2). Several factors may trigger this thermoregulatory dysfunction; for instance changes in the circulating concentrations of LH, FSH, estrogens, serotonin, norepinephrine, calcitonin gene-related peptide and neuropeptide Y may be involved (*Barton et al. 2001, Shanafelt et al. 2002, Whiteman et al. 2003, Rapkin 2007*). Fluctuations in estrogen levels that occur during the menopausal transition appear as one key factor of the disturbed thermoregulation (Figure 2). Although the impact of hypoestrogenism on hot flushes is indisputable, no clear associations between the levels of endogenous sex hormones and the presence or severity of hot flushes have been found (*Overlie et al. 2002, Whiteman et al. 2003, Randolph et al. 2005, Götmar et al. 2008, Santoro 2008*). In contrast, FSH elevations, which reflect the degree of hypoestrogenism, show clear correlations to hot flushes (*Randolph et al. 2005*).

The exact details of this thermoregulatory dysfunction in the hypothalamus are not understood, but a rise in core body temperature is somehow registered by the narrowed thermoregulatory zone in the hypothalamus. This launches heat-dispersal mechanisms, partly through changes in the autonomic nervous system, and results in cutaneous vasodilatation, flushing and sweating. These compensatory mechanisms may sometimes overreact, and chills ensue after hot flushes (Figure 2) (Freedman 2005, Deecher and Dorries 2007, Sturdee 2008).

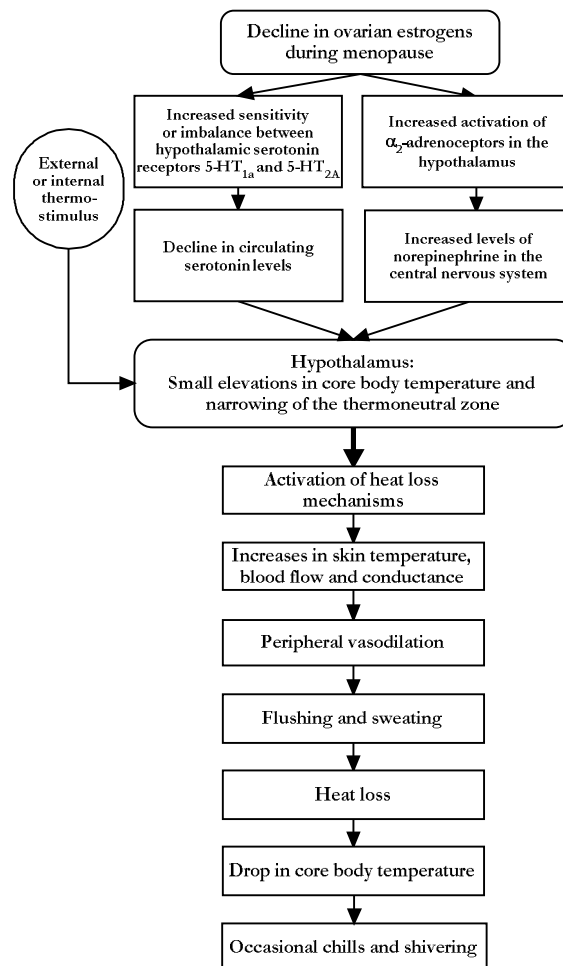


Figure 2. Possible physiological mechanisms for the initiation of hot flushes (*modified from Freedman 1998, Stearns et al. 2002, Freedman 2005, Deecher and Dorries 2007*).

Vasomotor symptoms can be reliably evaluated prospectively by using questionnaires in which both the frequency and severity of symptoms can be assessed (Table 1). One of the most established tests is the Hot Flush Weekly Weighted Score (HFWWS), in which mild symptoms are scored with 1, moderate symptoms are score 2, and severe symptoms are scored 3 (*Sloan et al. 2001, Loprinzi and Barton 2009*). With such a system it is possible to estimate the overall burden of hot flushes, because both their frequency and severity are accounted for. Recording hot flushes via such questionnaires mimics a clinical situation where a women herself must subjectively approximate the overall impact of hot flushes and decide on the possible initiation of HT. For pure research purposes, hot flushes have also been objectively quantified by measuring dermal temperature increase and/or changes in skin conductance (*Sievert 2007*). However, such tests are not very sensitive and are prone to many errors. Therefore, in clinical research, hot flushes are most commonly assessed by means of a subjective scale. This must be completed over a reasonably long period, usually for a minimum of 1–2 weeks, because hot flushes show relatively great day-to-day variation (*Sloan et al. 2001*).

Hot flushes	Definitions
Absent	No hot flushes
Mild	A transient sensation of heat without sweating
Moderate	Sensation of heat accompanied with sweating, but able to continue activities
Severe	Sensation of heat with sweating that causes cessation of activity

Table 1. Hot flushes defined by the United States Food and Drug Administration (*US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research*).



There are ethnic differences in hot flushes, and, for example, Asian women show a lower incidence of hot flushes (~16%) than Latin, European or African women (~47–57%) (*Green and Santoro 2009, Simpkins et al 2009*). Cultural differences have been presented as one explanation; it is possible that some cultures and languages lack definitions for hot flushes, or that women are reluctant to express their vasomotor symptoms (*Whiteman et al. 2003*). Furthermore, hot flushes also seem more prevalent in regions with large seasonal temperature changes (*Freeman and Sherif 2007, Sturdee 2008*).

Surgical menopause is defined as an oophorectomy in a fertile woman. This results in an abrupt decline in endogenous estrogen levels and often in severe hot flushes soon after surgery. Although endogenous estrogen levels show no consistent association with vasomotor symptoms (*Santoro 2008*), the presence of hot flushes has been linked with polymorphisms in estrogen-metabolizing cytochrome P450 enzymes (*Visvanathan et al. 2005, Crandall et al. 2006a, Woods et al. 2006, Schilling et al. 2007*), estrogen receptor alpha (ER- $\alpha$ ) (*Malacara et al. 2004*) and beta (ER- $\beta$ ) (*Takeo et al. 2005*), and genes that regulate angiogenesis (*Schneider et al. 2009*).

Hot flushes also accompany the use of antiestrogens such as tamoxifen, which is commonly used for the adjuvant treatment of breast cancer (*Deeher and Dorries 2007, Santoro 2008*). Smoking and alcohol use may predispose women to hot flushes (*Barton et al. 2001, Whiteman et al. 2003, Santoro 2008*). It has been thought that high BMI predisposes women to hot flushes as a result of the insulating effect of excess body fat (*Freeman and Sherif 2007*). On the other hand, the conversion of extraglandular androgens to estrogens in fatty tissues has been proposed to reduce hot flushes. However, BMI is not a clear-cut determinant of hot flushes, since both high and low BMI have been linked with hot flushes.

As evidenced above, menopausal hot flushes, deriving primarily from the central nervous system, affect many organs and significantly reduce the quality of life for several years in many postmenopausal women. Therefore, reduction of hot flushes has become the primary goal for modern treatment of menopausal symptoms.

## Cardiovascular disease

Cardiovascular disease is a major cause of morbidity and mortality in both men and women worldwide (*The World Health Organization 2007, Shaw et al 2009, van der Schoon 2009*). These diseases may manifest as angina, coronary heart disease (CHD), myocardial infarction, transient cerebral ischemic attacks and stroke (*The World Health Organization 2007*). Men and women differ in their cardiovascular risk profile, and women present with clinically significant disease approximately 10 years later than men (*Knopp 2002, Pepine et al. 2006*). However, over the past 20 years the overall cardiovascular risk profile in women has worsened (*Tonfighi et al. 2009*), and nowadays the mortality of women as a result of CVD exceeds that of men (*Shaw et al. 2009*).

It has been known for many decades that menopause is associated with an increase in the incidence of CVD (*Kannel et al. 1976, Witteman et al. 1989, Kannel and Wilson 1995, Dubey et al. 2005, Collins et al. 2007, Shaw et al. 2009*). There are many explanations for this phenomenon, but menopause-induced hypoestrogenism is the most established one (*Mendelsohn and Karas 1999, Collins 2001, Mendelsohn and Karas 2005*). Hypoestrogenism may lead to vascular inflammation, endothelial dysfunction and increased arterial stiffness, which all endanger vascular health (*Kingwell et al. 2001, Staessen et al. 2001, Takahashi et al. 2005, Zaydun et al. 2006*). It is also known that blood pressure (BP) increases in many women after menopause (*Zanchetti et al. 2005, Barton and Meyer 2009*), but the mechanisms behind this change are poorly understood (*Casiglia et al. 2008, Cifkova et al. 2008, Coylewright et al. 2008*); menopause-associated alterations in the function of the autonomic nervous system may in part contribute to the increase in BP (*Vongpatanasin 2009*). In addition, patterns of the lipid profile deviate in an atherogenic direction in many women during and after menopause (*Tikkanen 1996b, Rosano et al. 2007*). Furthermore, vasoactive prostacyclin, thromboxane and endothelins have been linked to the etiology of atherosclerosis alone or through effects on lipoproteins (*Ylikorkala et al. 1984, Ylikorkala et al. 1987, Mikkola et al. 1995, Ylikorkala et al. 1995, Mikkola et al. 1996*). These biochemical factors and hypoestrogenism-induced changes in them may predispose women to the development of CVD. It is further known that menopause is accompanied by android fat distribution and reduced glucose tolerance, in other words, features of metabolic syndrome; such changes are seen in up to 40–50% of postmenopausal Western women (*Kuh et al. 2005, Feng et al. 2008, Janssen et al. 2008, Lobo 2008*). The role of hypoestrogenism in CVD initiation gains further support from the data showing that premature menopause and consequent prolonged hypoestrogenism are associated with a particularly high risk of CVD (*Atsma et al. 2006, Archer 2009*).

## Additional menopausal features

Further symptoms attributed to the menopause include, for example, sleep disturbances, head and joint ache, palpitations, mood changes, oral discomfort and decreased libido (*Polo-Kantola et al. 2001, Utian 2005, Nelson 2008, Ensrud et al. 2009, Meurman et al. 2009, Nappi and Lachowsky 2009*). Many of these symptoms, such as broken sleep, tiredness or depressive mood may be secondary to hot flushes. It should also be mentioned that some women may be totally asymptomatic, but nevertheless, up 95% of women aged 52–56 years report at least one climacteric symptom (*Jokinen et al. 2003*). Long-term consequences of menopause include atrophy of the vaginal epithelium (*Greendale 1999, Santoro and Komi 2009*), whereas data on the correlation between decreasing estrogen levels and cognitive decline are controversial (*Barrett-Connor and Laughlin 2009*). The association between low estrogen levels and bone loss, however, is an established fact (*Wang et al. 2009*). It has been calculated that a women at 50 years of age is characterized by a 30–40% risk of bone fracture in her subsequent life; in absolute terms, increased bone loss and fracture risk after menopause are very significant determinants of overall quality and even length of life (*Huopio et al. 2000, Sirola et al. 2003, Rutanen and Ylikorkala 2004, The Finnish Medical Society and the Academy of Finland 2004*).

## Postmenopausal hormone therapy

Hormone therapy has been used for approximately 80 years to alleviate hot flushes (*Stefanick 2005*). The corner stone in HT is estrogen, which can be administered orally or transdermally. Estrogen alone (estrogen-only therapy; ET) can be prescribed to hysterectomized women. If a woman has an intact uterus, a progestagen must be added to oral or transdermal estrogen treatment (estrogen-progestagen therapy; EPT) to protect against endometrial hyperplasia (*Farquhar et al. 2009*). In Finland in 2008 various forms of HT were used by a total of 352 600 postmenopausal women (*Paakkari et al. 2008*). Contraindications for HT include a history of breast cancer or other hormone-dependent cancer, undiagnosed gynecological bleeding, thromboembolic disease, myocardial infarction, stroke, and uncontrolled hypertension (*The Finnish Medical Society and the Academy of Finland 2004*). Most liver diseases are cured successfully, and therefore, a sole history of liver disease is no contraindication for the use of HT. Thus, women with a history of intrahepatic cholestasis of pregnancy, for example, tolerate well the use of both oral and transdermal HT (*Ropponen et al. 2005a, Ropponen et al. 2005b, Tuomikoski et al. 2008*).

## *Estrogens*

Natural human estrogens are (17 $\beta$ )-estradiol, estrone and estriol; estradiol is the most potent of these. It binds to estrogen receptors  $\alpha$  and  $\beta$ , but it may also act directly via non-genomic mechanisms through cell membrane receptors (*Kuhl 2005*). In addition to the reproductive organs, estrogen receptors can be found in several tissues, e.g. in heart, blood vessels, bone, cartilage, skin and the central nervous system. Estrogens are carried in the blood stream bound to either sex hormone-binding globulin (SHBG) or albumin, and only 2% of circulating estrogens appear free. As a result of mutual conversion between estradiol and estrone, the concentrations of these estrogens in the blood are in equilibrium, and both are converted in the liver and gastrointestinal system to estriol. Various estrogens differ in their binding properties to estrogen receptors, and estrone has only about 4% of the estrogenic activity of estradiol (*Kuhl 2005*).

Estradiol is the drug of choice for menopausal complaints in Europe, whereas in the United States CEE is most commonly used. Conjugated equine estrogens, derived from the urine of pregnant mares, are a mixture of several estrogens, such as 17 $\alpha$ - and 17 $\beta$ -estradiol, estrone, equilin and equilenin. Therefore, the composition and even the biological effects of CEE may vary (*Kuhl 2005*).

Estetrol is a new steroid currently being investigated for the management of vasomotor symptoms. It was originally isolated from the fetal liver, and in rat models it shows both estrogen agonist and antagonist properties (*Coelingh Bennink et al 2008, Visser and Coelingh Bennink 2009*). So far there are no human data on the use of estetrol for the treatment of menopausal symptoms, but it may hold promise for this purpose.

## Progestagens

The use of unopposed estrogen in women with an intact uterus is associated with an increased risk of endometrial cancer (*Stefanick 2005, Furness et al. 2009*). Therefore, either a sequential progestagen at 10- to 14-day courses each month, or at three-month intervals (sequential EPT), or daily (continuous EPT) should be used in combination with estrogen (*Hickey et al. 2005*). Progestagens can be administered orally, transdermally, or through an intrauterine device (*Kuhl 2005*). A levonorgestrel-containing intrauterine device (*Niksson et al 1981, Luukkainen et al. 1986*) has proved to be an excellent treatment for menorrhagia (*Lähteenmäki et al. 1998, Hurskainen et al. 2001*), and it can also be used in postmenopausal women in combination with ET to ensure endometrial protection (*Peled et al 2007, Sitruk-Ware 2007a*). Progestagens differ in their affinity for steroid receptors and their biological activity also depends on their concentration in tissues (*Kuhl 2005*) (Table 2).

Progestagens	Estro- genic	Anti- estro- genic	Andro- genic	Anti- andro- genic	Gluco- corticoid	Anti- mineralo- corticoid
Progesterone	-	+	-	±	+	+
Dydrogesterone	-	+	-	-	-	-
<i>Progesterone-derivative</i>						
Medroxyprogesterone acetate	-	+	±	-	±	-
Megestrol acetate	-	+	±	+	+	-
<i>Testosterone-derivatives</i>						
Norethisterone	-	+	+	-	-	-
Levonorgestrel	-	+	+	-	±	-
Dienogest	±	±	-	+	-	-
<i>Spirolactone-derivative</i>						
Drospirenone	-	+	-	+	-	++

Table 2. Biological activity of various progestagens; (++) strong, (+) moderate, (±) weak, (-) none (*modified from Schindler et al. 2003, Kuhl 2005, Nath and Sitruk-Ware 2009*).

## Route of administration

Orally administered estrogens are subject to first-pass metabolism in the liver, unlike transdermally administered estradiol. Commonly, estradiol is given in valerate form in oral use and as hemihydrate in transdermal use; these agents may slightly affect the estrogenic bioprofiles (*Kuhl 2005*). Estradiol is given transdermally as a patch or as a gel; the latter is also called percutaneous form of administration. Oral and transdermal administration results in different estrogenic milieus; oral estradiol administration causes significantly higher circulating levels of estrone than does transdermal use of estradiol. The biological responses to oral and transdermal estradiol also differ, especially as regards liver-mediated responses (*Godsland 2001, Strandberg et al. 2003, Modena et al. 2005*) (Table 3). For instance, oral estrogen causes marked beneficial changes in lipids and lipoproteins, whereas lesser changes are seen in connection with transdermal estradiol treatment (with the exception of triglycerides) (*Tikkanen 1996b*). Markers of inflammation, such as C-reactive protein and adhesion molecules, and metalloproteinases, which affect atherosclerotic plaque stability, may be stimulated by oral estradiol, but not transdermal estradiol (Table 3) (*Kuhl 2005, Mueck and Seeger 2006, Ridker et al. 2008*). Use of oral estradiol may also increase the production of renin substrate from the liver leading to a consequent release of angiotensin-II with potential elevation of BP (*Mueck and Seeger 2004*) (Table 3). Furthermore, individual responses to oral and transdermal estradiol vary, both in terms of relief of vasomotor symptoms and increases in the circulating levels of estradiol (*Tuimala and Vihtamäki 1996, Vihtamäki and Tuimala 1998, Vihtamäki et al. 2004*). Thus, a clinician considering the most suitable form of HT for a given postmenopausal woman must be aware of these effects as well as the patient's disease history.

	Oral estradiol	Transdermal estradiol
Low-density lipoprotein cholesterol	↓↓	↓
Very low-density lipoprotein cholesterol	↑	↑
Apolipoprotein B	↓↓	↓
Lipoprotein (a)	↓↓	↓
High-density lipoprotein cholesterol	↑↑	↑
Apolipoprotein A	↑↑	↑
Triglycerides	↑↑	↔
Sex hormone-binding globulin	↑↑	↔
C-reactive protein	↑	↔
Adhesion molecules	↓	↔
Metalloproteinase activity	↑	↔
Renin substrate	↑↑	↔

Table 3. Differential effects of oral and transdermal estrogen on various biochemical markers; (↓) decrease, (↓↓) substantial decrease, (↑) increase, (↑↑) substantial increase, (↔) no effect (*modified from Kuhl 2005*).

## Effects of hormone therapy

### *Hot flushes*

Estradiol treatment, with or without a progestagen, is the most effective way to alleviate hot flushes. Oral and transdermal HT result in an approximate 75–95% reduction in hot flushes in a dose-dependent manner within 2–6 weeks, as demonstrated in several placebo-controlled studies, and the addition of daily or sequential progestagen does not affect this efficacy (MacLennan *et al.* 2004, Nelson 2004, Grady 2006). Recently, the focus has been on low-dose regimens; oral doses as low as 0.1–0.5 mg, or transdermal estradiol doses of 0.014 mg, can reduce hot flushes (Panay *et al.* 2007, van de Weijer *et al.* 2007, Langer 2009, Panay 2009). Progestagens, such as MPA, norethisterone and megestrol, when given without estrogen, may slightly alleviate hot flushes, but alone they are usually not used for the treatment of vasomotor symptoms (Hickey *et al.* 2005).

Hormone therapy at the lowest effective dose for healthy, recently postmenopausal women with disturbing moderate to severe hot flushes is an established treatment recommended by a number of expert organizations (Rutanen and Ylikorkala, 2004, *The Finnish Medical Society and the Academy of Finland* 2004, Utian *et al.* 2008, *International Menopause Society Consensus Statement* 2009). Cessation of HT may lead to reoccurrence of vasomotor symptoms in up to 50% of women, especially if hot flushes were moderate or severe at baseline, although the symptoms may be milder (Ockene *et al.* 2005, Ness *et al.* 2006, Lindb-Åstrand *et al.* 2009, Lindb-Åstrand *et al.* 2010). It is often recommended that the use of HT is stopped for 1–2 months every 2–3 years to evaluate whether hot flushes still occur, and if they do, HT can be continued (*The Finnish Medical Society and the Academy of Finland* 2004). The use of HT can be continued as long as it improves the quality of life, and therefore, there is no upper limit for the duration of use or the age of the woman (*The Finnish Medical Society and the Academy of Finland* 2004).

## Cardiovascular system

Because HT has been used for approximately 80 years, abundant clinical data on its effects on CVD have accumulated. These data can be considered as being in two categories; observational studies and randomized trials on HT. Approximately 40 observational or case-controlled studies show a 30–50% lower risk of CVD in users of HT, and therefore, until the late 1990s HT was commonly recommended for the prevention of CVD (*Colditz et al. 1987, Stampfer and Colditz 1991, Grady et al. 1992, Grodstein and Stampfer 1995, Barrett-Connor 1996, Hu and Grodstein 2002*) (Table 4).

	Population Follow-up	Main outcome measure	Results
<b>Sourander et al. (1998)</b>	n=7944 8 years	Cardiac death n=258	EPT: RR 0.21 (95% CI 0.08–0.59)
		Death due to stroke n=51	EPT: RR 0.16 (95% CI 0.02–1.18)
<b>Swedish cohort (Grodstein et al. 1999)</b>	n=9236 8 years	Myocardial infarction n=213	ET: RR 0.75 (95% CI 0.56–0.99) EPT: RR 0.69 (95% CI 0.45–0.90)
		Stroke n=289	ET: RR 0.91 (95% CI 0.71–1.17) EPT: RR 0.81 (95% CI 0.61–1.10)
<b>Varas- Lorenzo et al. (2000)</b>	n=164 769 5 years	Myocardial infarction n=1242	ET: OR 0.52 (95% CI 0.35–0.78) EPT: OR 0.79 (95% CI 0.59–1.08)
<b>Nurses’ Health Study (Grodstein et al 2000)</b>	n=70 533 20 years	Coronary heart disease n=1253	ET: RR 0.55 (95% CI 0.45–0.68) EPT: RR 0.64 (95% CI 0.49–0.85)
		Stroke n=767	ET: RR 1.18 (95% CI 0.95–1.46) EPT: RR 1.45 (95% CI 1.10–1.92)
<b>WHI Observatio- -nal study (Prentice et al. 2005)</b>	n=53 054 5 years	Coronary heart disease n=158	EPT: HR 0.87 (95% CI 0.72–1.05)
		Stroke n=123	EPT: HR 0.86 (95% CI 0.70–1.07)

Table 4. Outcomes of some of the largest observational or case-control studies done after 1995 in which the effects of long-term hormone therapy have been assessed. Abbreviations: CI: 95% confidence interval, ET: estrogen-only therapy, EPT: estrogen-progestagen therapy, HR: hazard ratio, OR: odds ratio, RR: risk ratio, WHI: Women’s Health Initiative.



The largest observational study was the Nurses' Health Study (*Grodstein et al. 2000*), which started in 1976. In this study 70 533 postmenopausal women were followed for 20 years and data were updated biannually. The data showed that current users of ET (current at the time) had a 45% reduction in the risk of CHD, which was similar as regards CEE at 0.3 mg (risk ratio [RR] 0.58, 95% confidence interval [CI] 0.37–0.92) and CEE at 0.625 mg (RR 0.54, 95% CI 0.44–0.67), and EPT (Table 4). However, the risk of stroke was increased by 45% in current users of EPT, and by 35% (RR 1.35, 95% CI 1.08–1.68) in ET users at a dose of 0.625 mg, whereas CEE at 0.3 mg did not confer any increase in the risk of stroke (RR 0.54, 95% CI 0.28–1.06) (*Grodstein et al. 2000*) (Table 4). The WHI trial also included an observational study arm consisting of 53 054 women of whom 17 503 (33%) were current EPT users (*Prentice et al. 2005*). During an average follow-up of 5.5 years (maximum 8.4 years) a non-significant protective effect was associated with current HT use (Table 4).

The protective effect of HT against CVD seen in observational studies has in part been attributed to the “healthy woman effect”, i.e. women who chose HT were in general healthier than women who did not use HT. Therefore, randomized, placebo-controlled trials, such as HERS (*Hulley et al. 1998, Grady et al. 2002*) and the WHI trial (*Rossouw et al. 2002, Manson et al. 2003, Anderson et al. 2004*) were undertaken. The benefits seen in observational studies were, however, not confirmed in these secondary or primary prevention trials, showing rather an increased risk of CVD (*Herrington and Klein 2003, Ylikorkala 2004*) (Table 5, a and b).

In the secondary prevention trial HERS the use of CEE at 0.625 mg and MPA at 2.5 mg for ~4.1 years in older women with established CHD was not associated with increased risk of coronary events (RR 0.99, 95% CI 0.80–1.22) or total mortality (RR 1.08, 95% CI 0.84–1.38). There was, however, a significant trend over time for more coronary events in HT users during the first years of use (relative hazard year I: 1.52; year II: 1.00; year III: 0.87; year IV: 0.67,  $p=0.009$  for trend) (*Hulley et al. 1998*). Follow-up after approximately 6.8 years showed that the lower rates of coronary events associated with prolonged HT use did not persist (RR 0.84, 95% CI 0.84–1.17) (*Grady et al. 2002*) (Table 5a).

Secondary prevention trials				
	Population	Treatment	Main outcome	Results
<b>HERS</b> 1998, 2002	n=2763–2321 mean age: 68 established CHD	4.1 to 6.8 years CEE 0.625 mg + MPA 2.5 mg	MI or death	No benefit or harm
<b>ERA</b> 2000	n=309 mean age: 66 established CHD	3.2 years CEE 0.625 mg ± MPA 2.5 mg	Angiography: change in coronary lumen diameter	No benefit or harm
<b>PHOREA</b> 2001	n=321 mean age: 59 CIMT ≥1mm	11 months oral estradiol 1 mg ± gestodene 0.025 mg	Ultrasound: change in CIMT	No benefit or harm
<b>PHASE</b> 2002	n=255 mean age: 66 established CHD	30.8 months transdermal estradiol 2mg ± 4mg cyclic NETA	Unstable angina, proven MI or cardiac death	No benefit or harm
<b>WAVE</b> 2002	n=423 mean age: 65 established CHD	2.8 years CEE 0.625 mg ± MPA 2.5 mg, and/ or vitamin C or E	Angiography: change in coronary lumen diameter	No benefit or harm
<b>ESPRIT</b> 2002	n=1 017 mean age: 62 previous MI	2 years oral estradiol 2mg	MI or death	No benefit or harm
<b>WELL- HART</b> 2003	n=226 mean age: 64 established CHD	3.3 years oral estradiol 1mg ± MPA 2.5 mg	Angiography: change in coronary stenosis	No benefit or harm
<b>WHISP</b> 2006	n=100 mean age: 69 acute coronary event <28 days	1 year oral estradiol 1 mg + NETA 0.5 mg	Death, MI, stroke, CVD admissions	Non- significant reduction in CVD events

Table 5a. Secondary prevention studies on hormone therapy. Abbreviations: CEE: conjugated equine estrogens, CHD: coronary heart disease, CVD: cardiovascular disease, CIMT: carotid intima-media thickness, MI: myocardial infarction, MPA: medroxy-progesterone acetate, NETA: norethindrone acetate, ±: with or without, +: with.

PHASE: Papworth HRT Atherosclerosis Study (*Clarke et al. 2002*), HERS I: Heart and Estrogen/progestin Study (*Hulley et al. 1998*), HERS II: Heart and Estrogen/progestin Study (*Grady et al. 2002*), ERA: Estrogen Replacement Atherosclerosis Trial (*Lakoski et al. 2005*), PHOREA: Postmenopausal Hormone Replacement against Atherosclerosis Trial (*Angerer et al. 2001*), WAVE: Women's Angiographic Vitamin and Estrogen Trial (*Waters et al. 2002*), ESPRIT: Estrogen in the Prevention of Reinfarction Trial (*Cherry et al. 2002*), WELL-HART: Women's Estrogen-Progestin Lipid-Lowering Hormone Atherosclerosis Regression Trial (*Hodis et al. 2003*), WHISP: Women's Hormone Intervention Secondary Prevention Study (*Collins et al. 2006*).

The WHI (CEE 0.625 mg with or without MPA) was designed as a primary prevention trial of CHD, breast and colorectal cancer and fractures in healthy, postmenopausal women between the ages 50–79. The trial was planned to continue for 8.5 years, but it was prematurely stopped at 5.2 years because of an increased risk for invasive breast cancer (7 extra cases/10 000 person-years), and the risks of CHD (7 extra cases/10 000 person-years) and stroke (8 extra cases/ 10 000 person-years) were also elevated (*Rossouw et al. 2002*). Interestingly, the use of ET in hysterectomized women was not associated with an increased risk of CHD (HR 0.91, 95% nominal CI 0.75–1.12) or breast cancer (HR 0.77, 95% nominal CI 0.59–1.01), whereas the risk of stroke was elevated (12 more cases/10 000 person-years) (*Anderson et al. 2004*) (Table 5b).

Primary prevention trials				
	Population	Treatment	Main outcome	Results
<b>EPAT</b> 2001	n=222 mean age: 62 LDL cholesterol >3.37mmol/L	2 years oral E2 1 mg $\pm$ statin	Ultrasound: change in CIMT	Similar decrease in IMT with ET $\pm$ statin treatment
<b>WHI-EP</b> 2002	n=16 608 mean age: 63	5.2 years CEE 0.625 mg + MPA 2.5 mg	CHD events	Hazard ratio 1.29 (nominal 95% CI 1.02–1.63)
			Stroke	Hazard ratio 1.41 (nominal 95% CI 1.07–1.85)
<b>WHI-E</b> 2004	n=10 739 mean age: 64	6.7 years CEE 0.625 mg	CHD events	Hazard ratio 0.91 (nominal 95% CI 0.75–1.12)
			Stroke	Hazard ratio 1.39 (nominal 95% CI 1.10–1.77)

Table 5b. Primary prevention studies on hormone therapy. Abbreviations: CEE: conjugated equine estrogens, CHD: coronary heart disease, CI: 95% confidence interval, E2: estradiol, ET: estrogen only; CIMT: carotid intima-media thickness, LDL: low-density lipoprotein cholesterol, MPA: medroxyprogesterone acetate,  $\pm$ : with or without, +: with.

EPAT: Estrogen in the Prevention of Atherosclerosis Trial (*Hodis et al. 2001*). WHI-EP: Women's Health Initiative [Estrogen and Progestin] (*Rossouw et al. 2002, Manson et al. 2003*). WHI-E: Women's Health Initiative [Estrogen only] (*Anderson et al. 2004*).

The WHI trial has received much criticism. For instance, the mean age of the participants in the EPT arm was 63 years, and 4.4% reported previous CHD, stroke or transient cerebral ischemia. In other words, they were hardly eligible subjects for a primary prevention trial. Moreover, hypertension (36%), smoking (10.5%), hypercholesterolemia (13%) and diabetes (4.4%) were common among the study subjects. In addition, 25% had previously used HT (Manson *et al.* 2003, Anderson *et al.* 2004). Thus, the primary prevention nature of the WHI trial may be disputed (Grodstein *et al.* 2003, Mikkola *et al.* 2004, Mikkola and Ylikorkala 2005).

Several plausible explanations have been suggested for the divergent findings between observational studies and randomized clinical trials regarding the cardiovascular benefit of HT. For instance, women who adhere to the given treatment, whether it is active or placebo, tend to have a more beneficial outcome than those with lower compliance to treatment. Differences in treatment regimens, ET versus EPT and the possible adverse effects of MPA, have also been discussed. Furthermore, the length of follow-up has been criticized; in observational studies this has been up to 10–20 years, whereas in randomized trials it has been only 5–7 years. On the other hand, observational studies can be criticized as regards failure to record carefully early clinical events. Time since menopause and a woman's age at the time of HT initiation have been highlighted as key explanatory factors for the discrepancy between results from observational studies and randomized trials. Advanced age of study subjects in randomized trials may well have been associated with subclinical atherosclerosis, which manifested during the first years of HT use, whereas the arteries in women in observational studies were considerably healthier and were additionally protected against atherosclerotic changes during HT use (Mikkola and Clarkson 2002, Waters *et al.* 2002, Grodstein *et al.* 2003, Herrington and Klein 2003, Mikkola *et al.* 2004, Mikkola and Ylikorkala 2005, van der Schoot and Grobbee 2005, Mikkola and Clarkson 2006).

## *Other organ systems*

Systemic HT affects the whole body, and therefore, all organs possessing estrogen receptors are affected by the treatment. The use of estrogen sustains bone structure and restores bone mineral density both of which deteriorate after the onset of menopause; the use of estrogen results in a reduction of osteoporotic fractures of up to 40–59% (*Wells et al. 2002, Farquhar et al. 2009*). It is also well established that estrogen use is associated with a reduction in the risk of colorectal cancer (*Hickey et al. 2005, Lethaby et al. 2008, Farquhar et al. 2009*). Most observational studies have shown a significant reduction (up to 40%) in the risk of Alzheimer's disease in the users of HT, but the randomized trials have failed to confirm this and in contrast showed an increase of the risk of (vascular) dementia (*Barrett-Connor and Laughlin 2009*). A two- to three-fold risk of venous thromboembolic events is associated with oral HT use (*Rachon and Teede 2008, Canonico and Scarabin 2009, Farquhar et al. 2009*), but this risk seems to be lower or even nonexistent as regards transdermal treatment (*Rachon and Teede 2008, van de Weijer 2008, Farquhar et al. 2009*).

Breast cancer is probably the most feared side effect of HT. The use of HT for over five years is associated with a 1.2- to 2.0-fold risk of breast cancer, being higher as regards EPT versus ET (*Lyytinen et al. 2006, Lyytinen et al. 2009a*), with a higher risk for continuous than sequential EPT (*Hickey et al. 2005, Lyytinen et al. 2009a, Lyytinen et al. 2009b*). Similarly, the use of estradiol and intrauterine progestagen administration has been linked to an elevated risk of breast cancer in a non-randomized follow-up study, but this risk elevation may be in part a result of selection bias; women with a higher risk of breast cancer may have been treated with an intrauterine progestagen-releasing device (*Lyytinen et al. 2010*). However, continuous EPT is associated with a 76% decrease in the risk for endometrial cancer (*Jaakkola et al. 2009*).

## Other treatment regimens

Although HT is the most effective treatment for hot flushes (*Nelson et al. 2006*), the increased risk of breast cancer associated with HT use has led to a search for alternative treatments.

Tibolone is a synthetic steroid, metabolized to isomers with estrogenic (3 $\alpha$ - and 3 $\beta$ -hydroxy-tibolone), progestagenic and androgenic ( $\Delta$ 4-tibolone) effects (*Kubl 2005, Notelovitz 2007*). Tibolone treatment is as effective as HT in reducing hot flushes, but it does not stimulate endometrial growth or increase mammographic breast density (*Kubl 2005, Notelovitz 2007*). However, the use of tibolone is accompanied by an increased risk of breast cancer (RR 1.36, CI 1.15–1.96, in Finnish women, for example) (*Lyytinen et al. 2010*). This elevation is of the same magnitude as that connected with ET. The androgenic properties of tibolone may enhance sexual desire, but it also reduces the level of high-density lipoprotein (HDL) cholesterol up to 20–30%, which has caused a concern regarding possible vascular risks associated with its use. At this stage it can only be stated that the vascular risk profile of tibolone is not fully explored (*Notelovitz 2007, Garefalakis and Hickey 2008, Jernman 2008*).

Selective estrogen receptor modulators (SERMs) have either agonistic or antagonistic estrogen effects in different tissues. In general, SERMs act as estrogen agonists on bone and the cardiovascular system, whereas they act antiestrogenically in the breast (*de Villiers 2009*). However, SERMs do not alleviate hot flushes, but instead may even aggravate or cause them (*Cano et al 2007, Shen and Stearns 2009*). There are a number of new SERMs under development, such as ospemifene and bazedoxifene (*Rutanen et al. 2003, Ylikorkala et al. 2003, Komi et al. 2005, Lobo et al. 2009, Pinkerton et al. 2009*). These compounds show curious organ specificity, and, for example, ospemifene strengthens the vaginal epithelium without having any effect on the endometrium or the breast (*Rutanen et al. 2003, Komi et al. 2005*). Thus, in future these compounds may be used as specific targeted therapies against a given menopausal symptom, such as atrophy of vaginal epithelium, but none of them have been approved for clinical use.

Nonsteroidal treatments include clonidine, selective serotonin and noradrenalin reuptake inhibitors, and gabapentin. They are all clearly less effective than estrogen in the treatment of hot flushes and also cause a number of side effects (*Suvanto-Launonen et al. 2005, Grady 2006, Nelson et al. 2006, Sturdee 2008, Shen and Stearns 2009*). Phytoestrogens may possess both estrogenic and antiestrogenic properties, and in some trials significant relief of vasomotor symptoms has been reported during their use (*Lethaby et al. 2007, Henderson et al.*

2008). However, placebo-controlled trials have failed to show the efficacy of phytoestrogens in reducing hot flushes (*Nikander et al. 2003, Lethaby et al. 2007, Henderson et al. 2008*). Herbal preparations, such as ginseng, evening primrose, dong quai, black cohosh, and vitamin E, have shown no or very modest efficacy against hot flushes (*Cheema et al. 2007, Shen and Stearns 2009*).

Acupuncture and yoga have been tested, but they show no consistent effect in alleviating vasomotor symptoms (*Cho and Whang 2009, Lee et al. 2009*). Physical exercise may slightly ameliorate hot flushes, perhaps by increasing hypothalamic and peripheral  $\beta$ -endorphin levels (*Daley et al. 2009*). In addition, life-style changes such as smoking cessation and reducing alcohol consumption, the use of fans, layered clothing and diet changes may subjectively somewhat alleviate hot flushes (*Barton et al. 2001, Santoro 2008, Shen and Stearns 2009*). Some data imply that certain relaxation techniques and paced respiration could also be helpful in the management of hot flushes (*Hickey et al. 2005, Santoro 2008, Shen and Stearns 2009*).

In summary, despite extensive research, there exists no truly effective non-steroidal treatment or non-pharmacological intervention to alleviate hot flushes.

# Assessment of cardiovascular health

It has been recognized that in women CVD is often not diagnosed on time, and even if it is, treatment is neglected (Collins *et al.* 2007, Mikkola 2009). Therefore, several guidelines for earlier diagnosis and for more active management of CVD in women have been constructed (D'Agostino *et al.* 2001, Conroy *et al.* 2003, Collins *et al.* 2007, Mosca *et al.* 2007). The essential feature in these guidelines is active assessment of CVD risk before the condition becomes clinically manifest. The risk of CVD is modified by several factors, all of which aid the identification of high-risk individuals; such women are targets of primary prevention of CVD (Table 6). Known risk factors alone may not be adequate tools for evaluating low-risk populations and more sensitive tools are needed, for instance, measurements of arterial stiffness and endothelial function. Recently, a multimarker approach that includes the evaluation of traditional risk factors in combination with assessment of vascular properties and biomarkers of CVD has been suggested as a useful method to assess the overall cardiovascular risk profile (Hamilton *et al.* 2007, Ikonomidis *et al.* 2008).

Factors that confer a high risk of cardiovascular disease in women	
Presence of $\geq 1$	Presence of $\geq 3$
Subclinical target organ damage:	cardiovascular risk factors:
Increased arterial stiffness, ankle-brachial index or carotid intima-media thickness	Hypercholesterolemia or hypertriglyceridemia
Impaired renal function	Smoking
Left ventricular hypertrophy	Elevated blood pressure
	Impaired glucose tolerance
	Abdominal obesity
Independent risk factors:	Age $>65$ years
Hypertension, renal disease, diabetes, metabolic syndrome	Family history of cardiovascular disease: women at age $<65$ years men at age $<55$ years
Additional risk factors:	
unhealthy diet, physical inactivity, excessive alcohol consumption, low socioeconomic status, stress, depression	

Table 6. Risk factors of cardiovascular disease in women (modified from Mancia *et al.* 2007, Mosca *et al.* 2007, Ritz 2007, The World Health Organization 2007, Evangelista and McLaughlin 2009, Schenck-Gustafsson 2009, Shaw *et al.* 2009).



## Vascular function

The endothelium consists of the inner layer of cells on the blood vessel wall, which secretes several substances that regulate vascular tone and growth, and modulate inflammation, platelet aggregation and coagulation (Koh 2002, Munzel et al. 2008). Vascular tone may be in part controlled by the balance between vasodilatory nitric oxide and the vasoconstrictor endothelin-1 secreted by the endothelium (Ylikorkala et al. 1998, Koh 2002, Lane et al. 2006, Patel and Arora 2008). Hypertension, hypercholesterolemia and hypoestrogenism impair endothelial function thus leading to enhanced inflammatory reactions, oxidation of lipoproteins, proliferation of vascular smooth muscle cells, changes in extracellular matrix constitution, accumulation of lipid-rich material and prothrombotic changes (Koh 2002); all these are early steps in the development of atherosclerosis. Endothelial dysfunction may also increase arterial stiffness and BP by inducing vasoconstriction (Lane et al. 2006, Wang et al. 2008). Thus, endothelial dysfunction is a predisposing factor as regards CVD and it also contributes to the clinical progression of these diseases (Lane et al. 2006, Munzel et al. 2008, Rachen and Teede 2008, Wang et al. 2008) (Figure 3).

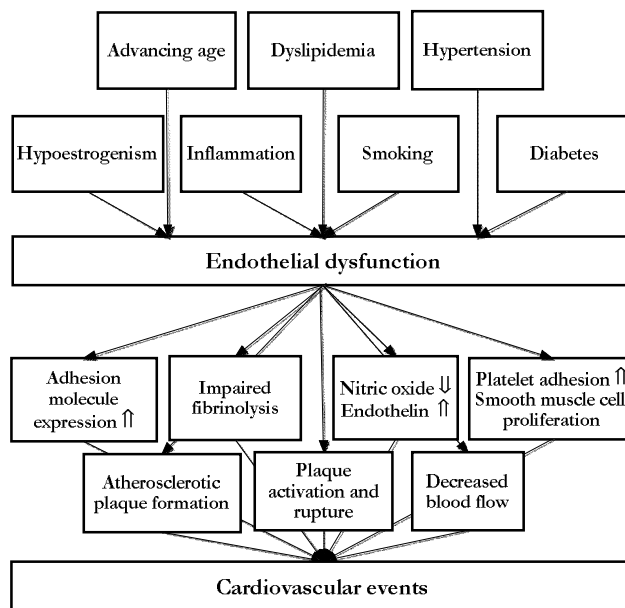


Figure 3. Schematic presentation of factors that cause endothelial dysfunction, and its consequences (modified from Strandberg and Tikkanen 1996, Ylikorkala et al. 1998, Mikkola and Clarkson 2002).

Endothelial function can be assessed, for example, by evaluating the ability of a given blood vessel to respond to vasodilatory substances, such as salbutamol and acetylcholine, or to reactive hyperemia, all of which cause release of nitric oxide from the endothelium (*Patel and Arora 2008*). This endothelium-dependent vasodilation can be compared with endothelium-independent vasodilation, which can be triggered by the administration of nitroglycerin, which mimics the action of nitric oxide (*Sarabi et al. 2001, Hayward et al. 2002, Wilkinson et al. 2002a, McEniery et al. 2006*). The dilatory capacity of a vessel can be measured, for instance, by applanation tonometry (pulse wave analysis; PWA) and ultrasonography (flow-mediated dilation; FMD) of a distal artery, and finger-pulse plethysmography (*Conroy et al. 2003, Kullo and Malik 2007*). Intracoronary infusion of vasodilatory substances and subsequent quantitative coronary angiography is the gold standard of endothelial testing; this technique, however, is both invasive and expensive (*Patel and Arora 2008*). Both PWA and FMD are noninvasive, but with PWA technology the validity of measurements is controlled by a quality index calculated by the software. Additional ways to assess endothelial function include measuring biomarkers reflecting vascular injury, such as cytokines, inflammation markers, circulating endothelial cells and microparticles, and markers of oxidative stress (*Lane et al. 2006, Munzel et al. 2008*).

In addition to endothelial function, blood flow in the arteries is controlled by the stiffness of the vessels, which contributes both to the velocity of the pulse wave and that of the waves reflected back from the periphery towards the heart. The loss of vascular elasticity due to aging, arteriosclerosis, hypertension or other vascular debilitating factor enhances arterial stiffness. This results in an increase in the speed of the forward pressure wave, and amplification of the pulse pressure by the reflected wave (*Mitchell et al. 2004, Zoungas and Asmar 2007, Wang et al. 2008*). Increased arterial stiffness may be associated with endothelial dysfunction, alterations in vascular wall matrix proteins, proliferation of smooth muscle cells, inflammation, and genetic determinants may also be involved (*Laurent and Boutouyrie 2007, Wang et al. 2008*). Arterial stiffness can be assessed, for example, by measuring pulse transit time (pulse wave velocity), changes in vessel diameter (distensibility), or amplification of the pulse pressure caused by the reflected waveform, for instance PWA. Measurement of arterial stiffness, either as a surrogate end-point or therapeutic target, has been used in both epidemiological studies and clinical trials (*Davies and Struthers 2003, Hamilton et al. 2007, Zoungas and Asmar 2007, Safar 2008, Wang et al. 2008*), and it is a valuable tool for the assessment of vascular health in individuals with little or no end-organ disease (*Zoungas and Asmar 2007*).

## *Blood pressure*

Elevated BP is one of the most important cardiovascular risk factors in both men and women (Ritz 2007, Barton and Meyer 2009, Evangelista and McLaughlin 2009). Blood pressure is considered normal if it is  $<120/80$  mmHg, and hypertensive if it is  $\geq 140/90$  mmHg (Mancia *et al.* 2007). However, it is conspicuous that BP without a threshold, for instance, high normal (130-139/85-89 mmHg), confers an increased risk of CVD (Vasan *et al.* 2001, Lewington *et al.* 2002, Svetkey 2005, Kshirsagar *et al.* 2006, Conen *et al.* 2007).

Blood pressure is normally measured manually with a mercury sphygmomanometer. This is prone to many errors of which the so-called “white coat hypertension” effect is the most common. Therefore, for research purposes BP should be measured by means of ambulatory monitoring over 24 hours. Ambulatory BP registration enables the assessment of diurnal changes and variability in BP that are undetectable with standard BP registrations (Pickering *et al.* 2006, Mancia *et al.* 2007). Moreover, ambulatory BP can reveal blunted nocturnal falls in BP, which are known cardiovascular risk factors (Verdecchia *et al.* 2007). In clinical trials ambulatory BP is considered the gold standard of assessing BP, since this technique enables an accurate estimation of 24-hour BPs, and separate analyses of both day- and night time BPs (Pickering *et al.* 2006).

## *Biochemical markers*

The levels of sex hormones have a profound impact on vascular health, as reviewed earlier. This concerns not only estrogens, but also progestagens and androgens, although their impact on cardiovascular health is less clear (Schindler *et al.* 2003, Dubey *et al.* 2005, Karim *et al.* 2008, Onyang *et al.* 2009). A large body of data shows that low levels of SHBG may indicate an elevated risk of CVD in women (Davison and Davis 2003, Rexrode *et al.* 2003, Karim *et al.* 2008, Sowers *et al.* 2008). This association may be related to differences in endogenous estrogen or androgen levels, or insulin resistance (Davison and Davis 2003, Sutton-Tyrrell *et al.* 2005, Crandall *et al.* 2006b, Joffe *et al.* 2006, Castela and Gago-Dominguez 2008), since the SHBG molecule itself is hardly a determinant of vascular health.

In addition to impaired glucose tolerance (*Evangelista and McLaughlin 2009*), proatherogenic changes, such as elevations in total cholesterol, low-density lipoprotein cholesterol (LDL) or triglycerides, or a reduction in HDL can predict the risk of CVD in women (*Ridker et al. 2000, Knopp 2002*). Further lipid-based CVD markers include apolipoprotein B (apoB) and A-I (apoA-I), and their ratio, as well as lipoprotein a [Lp (a)] (*Dahlen 1994, Davison and Davis 2003, Carmena et al. 2004, Chan and Watts 2006, Walldius and Jungner 2006, Suk Danik et al. 2008*). Apolipoprotein B molecules are present in very low-density lipoprotein cholesterol (VLDL), LDL and Lp (a) and thus the level of apoB indirectly reflects the total load of circulating atherogenic lipoproteins. In contrast, apoA-I is present only in HDL particles, thus representing cardioprotective lipoproteins (*Davison and Davis 2003, Chan and Watts 2006*).

Inflammation is a more recently discovered determinant of atherosclerosis and an elevated risk of CVD (*Lind 2003*). It can be detected, for example, by measuring high-sensitivity C-reactive protein (hs-CRP), fibrinogen, interleukine-6, monocyte-attracting protein-1 and adhesion molecules. Of these, hs-CRP is the most established marker (*Ridker et al. 2000, Davison and Davis 2003, Lind 2003, Muecke and Seeger 2006, Wang et al. 2006, Musunuru et al. 2008, Packard and Libby 2008*). Plasminogen-activator inhibitor-1 and matrix metalloproteinase-1, in turn, affect atherosclerotic plaque stability and may predict occlusive vascular events (*Muecke and Seeger 2006*).

## AIMS OF THE STUDY

The present study was designed to assess the impact of hot flushes on vascular health variables in recently postmenopausal women before and during various forms of HT.

The specific aims were to compare:

- Vascular function (Study I), 24-hour ambulatory BP (Study II) and vascular biomarkers (Study III) in women with no, mild, moderate or severe hot flushes
- Responses of the above-mentioned variables (Studies IV–VI) to oral and transdermal HT in women with tolerable or intolerable hot flushes

## SUBJECTS AND STUDY DESIGN

This study was approved by Helsinki University Women's Hospital Ethics Committee (no. 329/E8/03) and registered with the National Agency for Medicine (EudraCT 2004–005091–16) and the U.S. National Institutes of Health Clinical Research Registry (no. NCT00668603). The study was conducted according to the principles of Good Clinical Practice and the Declaration of Helsinki, and written informed consent was obtained from all participants.

The volunteers were recruited via local newspapers between May 2005 and May 2007. Nearly 1500 responding women were first interviewed over the telephone by a trained research nurse. Four hundred women who met our inclusion criteria (age between 48–55 years, last menstrual period within the preceding 6–36 months, no previous HT use) were invited to record their vasomotor symptoms for two weeks on a structured questionnaire that rated the symptoms according to their number and severity (*Panay et al. 2007*).

Hot flushes were defined as mild if there was only a slight sensation of heat which did not disturb daily life. A moderate hot flush was defined as more intense sensation of heat while awake with some perspiration. A severe hot flush was defined as being more intense with profuse sweating that clearly interfered with daily life or with sleep (*Panay et al. 2007*). Hot flushes were then rated by using the HFWWS, which scores mild symptoms as 1, moderate symptoms as 2 and severe symptoms as 3 (*Notelovitz et al. 2000, Sloan et al. 2001, Panay et al 2007*) (Table 7).

Hot flushes	Definitions	Score	HFWWS
Absent	No hot flushes		0
Mild	A slight sensation of heat which did not disturb daily life	1	0.5–9.5
Moderate	Intense sensation of heat while awake accompanied by some perspiration	2	10.0–99.5
Severe	Intense sensation of heat with profuse sweating that clearly interfered with daily life or sleep	3	100.0–452.5

Table 7. Definitions of hot flushes of different degrees of severity.  
HFWWS=Hot Flush Weekly Weighted Score.

To be able to accurately distinguish the possible impact of hot flushes on vascular health we chose to exclude women with only “intermediate” hot flushes, i.e.  $\geq 3$  mild hot flushes to  $\leq 7$  moderate to severe hot flushes/day. Intermediate hot flushes, smoking, chronic illness (such as diabetes or hypertension [BP  $>140/90$  mmHg]) or use of prescription drugs (including current or previous use of HT) were the main causes for the exclusion of 1100 subjects. Further exclusion criteria were a level of FSH  $<30$  U/L, BMI  $>30$  kg/m<sup>2</sup>, hysterectomy, ovariectomy, and unwillingness or inability to comply with the study plan. Thus, 150 women were eligible for this study (Figure 4).

For Studies I, II and III the women were classified (based on the HFWWS) as having mild, moderate or severe hot flushes, or as being totally asymptomatic (Table 7, Figure 4). At baseline 143 women were available for PWA (Study I). Due to hypertensive readings (BP  $>140/90$  mmHg) in the ambulatory BP recordings, three women out of 150 were excluded from further analysis of ambulatory BP and thus, this study group consisted of 147 women (Study II). One hundred and fifty women were eligible for the analysis of vascular biomarkers for CVD (Study III).

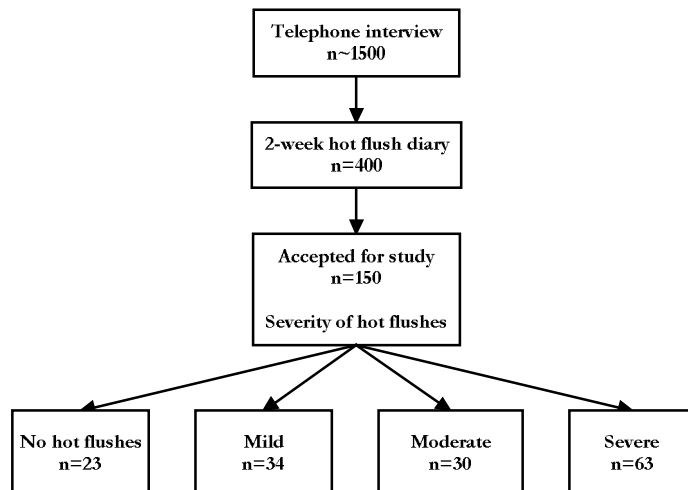


Figure 4. Gathering of the population for studies on the impact of hot flushes on various cardiovascular risk factors (Studies I, II and III).

For the randomized controlled trials on the impact of hot flushes on the responses of CVD risk factors to HT (Studies IV, V and VI) the women were classified as having either intolerable ( $\geq 7$  moderate/severe symptoms per day) or tolerable hot flushes ( $\leq 3$  mild symptoms per day). The women were randomized in blocks of four with regard to hot flush status (intolerable or tolerable hot flushes) to receive, in a double blind setting, either 1 mg of transdermal estradiol hemihydrate gel, 2 mg of oral estradiol valerate with or without daily MPA at 5 mg, or placebo for six months; these regimens were provided by Orion Pharma (Espoo, Finland) (Figure 5). All the women used both tablets (active or placebo) and gels (placebo or active) to guarantee the double-blind character of the trial. Compliance was assessed by counting the unused packages. As a safety precaution, each woman showing endometrial thickness of  $\geq 9$  mm at six months received MPA (5 mg/day) for two weeks to initiate endometrial shedding; this was started after collection of blood samples and all relevant clinical data. In this setting the impact of hot flushes on the responses of arterial stiffness and endothelial function (Study IV), ambulatory BP (Study V) and biochemical markers for CVD (Study VI) to various forms of HT was studied.

On the whole, a very limited number of participants discontinued the trial. Depending on the study a total of 6 to 9 women discontinued for the following reasons: withdrawal of consent, becoming lost during follow-up, or because of spotting.

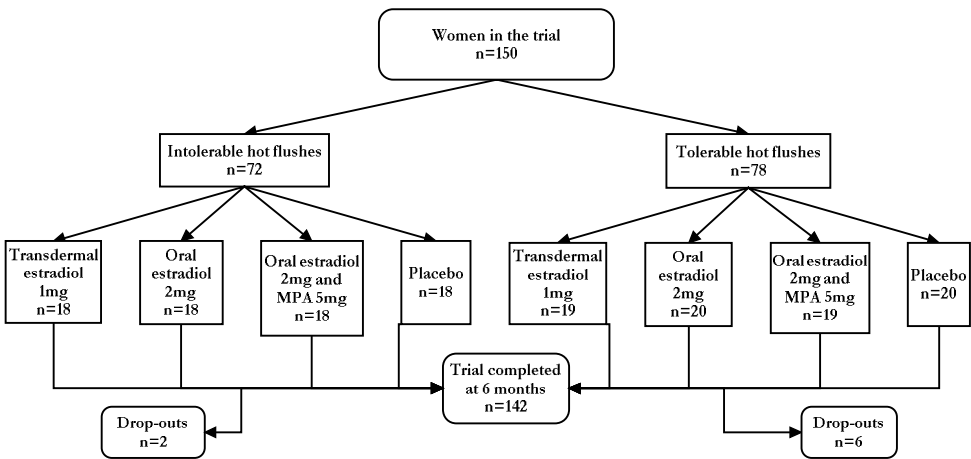


Figure 5. Study protocol for the randomized, placebo-controlled HT Studies IV, V and VI.



# METHODS

All participants underwent a thorough physical examination, and findings in pelvic and vaginal ultrasonographic examination, Papanicolaou smears and endometrial biopsy samples were normal.

## Pulse wave analysis and endothelial testing

For Studies I and VI we used PWA (SphygmoCor® 7.0, AtCor Medical, Sydney, Australia). In PWA central aortic waveforms are derived from a series of peripheral pulse waves that are recorded at the radial artery with a tonometer. The central aortic pulse wave is then calculated using a generalized transfer function, based on the combination of the forward-moving pressure wave generated by left ventricular systole, and waves reflected back to the aorta from points of greater impedance along the arterial tree, such as bifurcations and arterioles (O'Rourke *et al.* 2001, Mackenzie *et al.* 2002, Wilkinson *et al.* 2002a, O'Rourke and Adji 2005).

Systemic arterial stiffness was assessed with the augmentation index (AIx), defined as the difference between the second and the first systolic peaks expressed as a percentage of pulse pressure (O'Rourke *et al.* 2001, Mackenzie *et al.* 2002, Wilkinson *et al.* 2002a, O'Rourke and Adji 2005). Aortic stiffness was measured from the inflection point after the first systolic peak, which marks the time to the return of the reflected wave, dependent on pulse wave velocity, and it can therefore be used as a marker of aortic stiffness (Margo *et al.* 1980, London *et al.* 1992, Marchais *et al.* 1993, Wilkinson *et al.* 2001). Furthermore, left ventricular properties were estimated from the aortic pulse wave, by measuring the amplitude and timing of the first systolic peak, which are dependent on stroke volume and the rapid phase of ventricular ejection, respectively (Wilkinson *et al.* 2002b) (Figure 6, Table 8).

In the case of increased arterial stiffness, a rise in AIx and a decrease in time to the reflected waveform are seen, indicating increased augmentation and a faster return of the reflected wave, respectively (Zoungas and Asmar 2007). Since AIx is influenced by heart rate, it was normalized to a heart rate of 75beats/min for comparisons (Wilkinson *et al.* 2000, Wilkinson *et al.* 2002b). For the final analyses the lowest AIx value of three consecutive measurements was selected for each patient (Sarabi *et al.* 2001, McEniery *et al.* 2006, Törmälä *et al.* 2008).

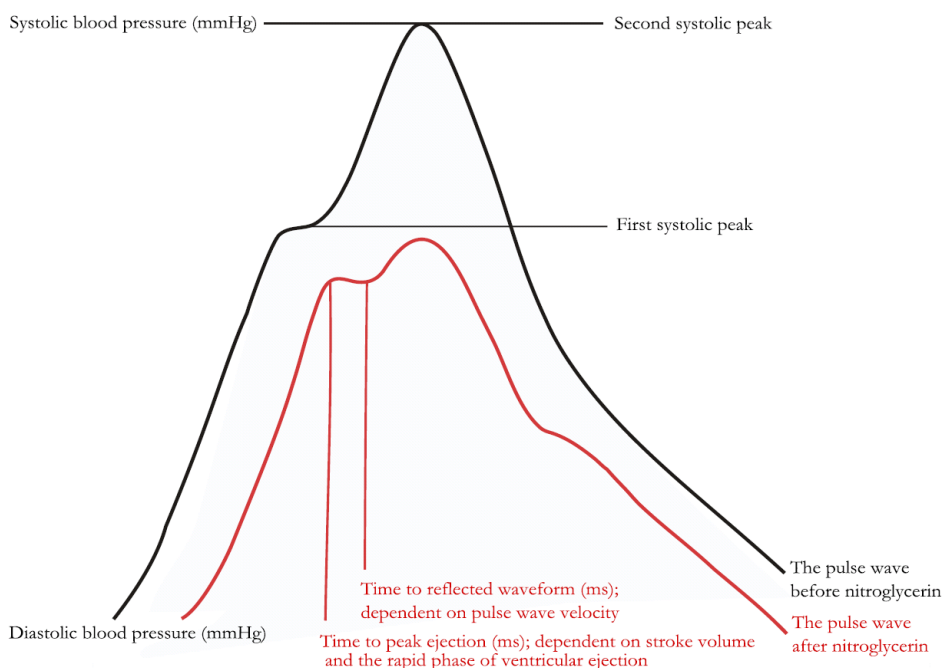


Figure 6. Schematic presentation of the pulse wave before and after nitroglycerin challenge (Study I). Reprinted and slightly modified with permission from Wolters Kluwer Health.

Variables in pulse wave analysis	Definitions:
Augmentation Index (AIx)	$AIx = \frac{P2 - P1}{SBP - DBP} * 100\%$ <p>The difference between the 2<sup>nd</sup> and the 1<sup>st</sup> systolic peaks expressed as a percentage of pulse pressure (systolic BP – diastolic BP)</p>
Endothelial Function Index (EFI)	$EFI = \frac{AIx \text{ before Salbutamol} - AIx \text{ after Salbutamol}}{AIx \text{ before Nitroglycerin} - AIx \text{ after Nitroglycerin}} * 100\%$ <p>Endothelium-dependent vasodilation expressed as a percentage of endothelium-independent vasodilation</p>
Time to peak ejection in milliseconds (T <sub>e</sub> )	Timing of the first systolic peak; dependent on stroke volume and the rapid phase of ventricular ejection
Time to reflected waveform in milliseconds (TR)	The time to the return of the reflected wave; dependent on pulse wave velocity

Table 8. Definitions of variables derived from pulse wave analysis.

Endothelial function was assessed by complementing PWA with nitroglycerin (causes endothelium-independent vasodilation) and salbutamol (causes endothelium-dependent dilation) challenges, and calculating the endothelial function index (EFI) (Figure 6, Table 8). The AIx measurements were carried out before and 3, 5, 10, 15 and 20 minutes after the administration of 0.5 mg of sublingual glyceryl trinitrate (Nitro<sup>®</sup>, Orion Pharma, Espoo, Finland). After a 60-minute washout period and a second baseline assessment, the measurements were repeated 5, 10, 15 and 20 minutes after inhalation of 0.4 mg salbutamol (Ventoline<sup>®</sup> Evohaler, GlaxoSmithKline, Ware, UK) (*Sarabi et al. 2001, Hayward et al. 2002, Wilkinson et al. 2002a, McEniery et al. 2006, Rönnback et al. 2007, Törmälä et al. 2008*).

## Ambulatory blood pressure

The participants wore calibrated oscillometric ambulatory BP monitors (SpaceLabs 90217, SpaceLabs Healthcare, Washington, U.S.A.) on their non-dominant arm for 24 hours (Studies II and V). The monitor was programmed to take readings at 20-minute intervals during periods of wakefulness and at 30-minute intervals during sleep according to each participant's estimated bedtime. The women were instructed to maintain their normal daily activities during the ambulatory BP recordings, and to record their activities and vasomotor symptoms in a diary. States of wakefulness and sleep state were defined according to diary entries.

All successful measurements of systolic and diastolic BP, together with simultaneous heart rate and the exact time of the measurement were transferred from the ambulatory BP system to a spreadsheet for further analysis of 24-h BP and heart rate, and separate analyses for daytime and night-time. Pulse pressure was defined as systolic minus diastolic BP, and absolute dipping was defined as the difference between average systolic BPs awake and asleep. Relative dipping was calculated as the percentage of absolute dipping of systolic BP when awake, and relative dipping of at least 10% was considered a normal dipping pattern (*Pickering et al. 2006*). The acute effects of hot flushes on BP were analyzed from night-time measurements in women with severe hot flushes. Diaries were used to identify the exact time of the hot flush. A recording occurring within 15 minutes after a hot flush was defined as representing the BP and heart rate during and after a hot flush, and the data were compared with the average BP and heart rate at night-time. We were able to identify 44 such hot flush episodes among 26 women, and in case of multiple hot flush experiences the mean value per subject was included.

## Assays for biochemical markers for cardiovascular disease

Fasting blood samples were collected and serum was separated with centrifugation. Samples at 6 months were scheduled to be collected approximately two hours after the intake of the study medication. Serum samples, kept frozen in -80°C, were assayed for total cholesterol, HDL, LDL, VLDL and triglycerides using standard methods (*Havel et al. 1955*). Commercially available kits were employed for apoB, apoA-I and Lp (a), SHBG and hs-CRP and estradiol. The level of estrone was assayed by liquid chromatography-tandem mass spectrometry based on the method of Nelson et al (*2004*) with some modifications (Study III). The free estradiol index (FEI) was calculated as the ratio: estradiol (nmol/L) x100/SHBG (nmol/L) (Table 9). Thyroid-stimulating hormone was tested using chemiluminescent microparticle immunoassay (Abbott Diagnostics, Abbott Park, IL) and FSH was analyzed by time resolved immunofluorometric assay method (AutoDelfia™, Wallac, Turku, Finland) according to the manufacturer's instructions.

Analyte	Source	Principle of assay	Coefficient of variation		Detection limit
			Intra-assay	Interassay	
Total cholesterol	Thermo Electron Co Konelab™ Vantaa, Finland	Enzymatic colorimetry	0.9–1.1%*	0.9–2.0%*	0.1 mmol/L
High-density, low-density, and very low-density lipoprotein cholesterol		Ultracentrifugation	0.42–1.3%*	1.5–2.2%*	0.084 mmol/L
Triglycerides	Thermo Electron Co Konelab™ Vantaa, Finland	Enzymatic colorimetry	1.0–1.9%*	2.0–2.5%*	0.02 mmol/L
Lipoprotein (a)	Thermo Electron Co Konelab™ Vantaa, Finland	Immunoprecipitation	1.3–2.6%*	2.7–3.5%*	30 mg/L
Apolipoprotein B	Thermo Electron Co Konelab™ Vantaa, Finland	Immunoprecipitation	1.6–1.7%*	2.8–3.4%*	50 mg/L
Apolipoprotein A-I	Thermo Electron Co Konelab™ Vantaa, Finland	Immunoprecipitation	2.7%	5.5%	100 mg/L
Sex hormone-binding globulin	AutoDELFIA™, Wallac, Turku, Finland	Immunofluorometry	1.4–1.8%*	8.2–10.1%*	0.5 nmol/L
High-sensitivity C-reactive protein	Orion Diagnostica Oy Espoo, Finland	Immunoturbidimetry	0.3–6.6%*	2.1–11.9%*	0.25 mg/L
Estrone	CDN Isotopes Inc. Pointe-Claire, Canada	Liquid chromatography-tandem mass spectrometry	<7.8–12.0%*	<7.8–12.0%*	10 pmol/L
Estradiol	Diasorin Inc. Stillwater, MN, USA	Radioimmunoassay	<6.1%	<6.1%	19 pmol/L

Table 9. Characteristics of the assays used for the measurement of biochemical markers of cardiovascular disease. \*=for low and high concentrations, respectively.

# Statistical analyses

Power analysis was made with the anticipated mean change in PWA in response to HT estimated to be 6.6% with a standard deviation (SD) of 1. The comparisons were calculated to achieve 80% power to detect a 15% difference in the responses to HT between women with intolerable and tolerable hot flushes at a conventional alpha-level of 0.05. The responses in biochemical markers and ambulatory BP were secondary endpoints of this trial.

Normality was assessed with the Shapiro–Wilk test. Logarithmic transformations and inverse transformation were made to achieve normal distribution, or the data was converted to ranked cases. Other statistical methods used are presented in Table 10.

One-way between-groups comparisons	Within- groups comparisons	Test for trend	Association between variables	Two-way between-groups comparisons
Parametric tests for normally distributed variables				
One-way analysis of covariance	Analysis of variance for repeated measures		Pearson's product- moment correlation coefficient	Two-way between groups analysis of covariance with Tukey HSD and Games– Howell <i>post hoc</i> comparisons
Linear regression analysis				
Non-parametric tests for variables with non-normal distribution				
Kruskall–Wallis test followed by Mann–Whitney U test with Bonferroni corrections	Wilcoxon's signed ranks test	Jonckheere– Terpstra test for trend	Spearman's nonparametric correlation coefficient	
			Chi-square test	

Table 10. Statistical methods used in the data analyses.

When appropriate, analyses were conducted adjusting for the time since menopause and the levels of estrone, estradiol and the FEI. Owing to multicollinearity between variables, a univariable instead of a multivariable approach was used (Studies IV and V) and partial  $\eta^2$  ( $\eta^2$ ) was calculated to describe the proportion of variance in the variables explained by treatment. Tukey HSD and Games–Howell *post hoc* comparisons were used for variables with equal and unequal variances, respectively. Due to significant interaction between hot flush status and the effect of treatment in Studies IV and V separate analyses were carried out for women with intolerable and tolerable hot flushes (Study IV), and the different treatment groups (Study V). A *p*-value <0.05 was considered statistically significant. All data are presented as mean $\pm$ SD or mean $\pm$ standard error of the mean (SEM). All analyses were carried out with SPSS software for Windows version 14.0, 2005 (SPSS Inc. Chicago, IL, USA).

## RESULTS

Detailed results are given in the original publications and only the main results are presented here.

### Impact of hot flushes on vascular health

Women with no, mild, moderate, or severe hot flushes were comparable in age, weight, height, BMI, and the level of thyroid-stimulating hormone. Importantly, there were no significant differences in the serum levels of estrone, estradiol, or the FEI. A significant trend was observed for the time since the last menstruation to be shorter with increasing severity of hot flushes, being approximately 6 months between asymptomatic women and those with severe hot flushes (Study I  $p=0.006$ ; Study II  $p=0.004$ ; Study III  $p=0.006$  [ $p$ -for-trend]).

#### *Vascular function (Study I)*

In PWA after the challenge with nitroglycerin, time to peak ejection was 12.3% ( $p=0.025$ ) longer and time to reflected waveform was 9.2% ( $p=0.014$ ) longer in women with severe hot flushes as compared with asymptomatic women. A significant trend across the groups was also detected; both time to peak ejection and time to reflected waveform after nitroglycerin were longer with increasing severity of hot flushes ( $p=0.001$  and  $p=0.002$ , respectively). No effect of salbutamol was seen in any of the study groups. Hot flush status did not influence arterial (AIx) or aortic stiffness (time to reflected waveform), or endothelial function (EFI) (Table 11).

Hot flushes	None	Mild	Moderate	Severe	<i>p</i> -value
Augmentation index	23.7±1.7	22.6±1.4	22.0±1.3	22.1±1.1	ns
Endothelial function index	21.3±11.2	37.7±16.1	24.7±6.2	26.7±5.2	ns
Time to peak ejection at baseline	106.5±2.1	110.1±2.2	113.0±2.2	107.7±2.0	ns
Time to peak ejection after nitroglycerin	115.5±3.5	120.2±3.7	127.6±4.6	131.5±3.4	0.025 †; 0.001 ‡
Time to peak ejection after salbutamol	109.1±3.0	106.9±1.7	112.8±2.7	110.7±1.8	ns
Time to reflected waveform at baseline	138.9±2.2	143.2±2.2	145.3±1.7	141.4±2.1	ns
Time to reflected waveform after nitroglycerin	149.8±2.2	157.6±3.2	159.4±3.3	164.0±2.6	0.014 †; 0.002 ‡
Time to reflected waveform after salbutamol	143.9±2.4	142.5±1.7	147.6±2.5	145.6±1.7	ns

Table 11. Variables in pulse wave analysis before and after challenges with nitroglycerin and salbutamol. ns=non-significant, †=*p* for the difference between asymptomatic women and women with severe hot flushes, ‡=*p* for trend across the groups. Data presented as mean ± SEM.



## *Ambulatory blood pressure (Study II)*

Women with different severity of hot flushes were fully comparable in their 24-hour BP and heart rate profiles (Figure 7).

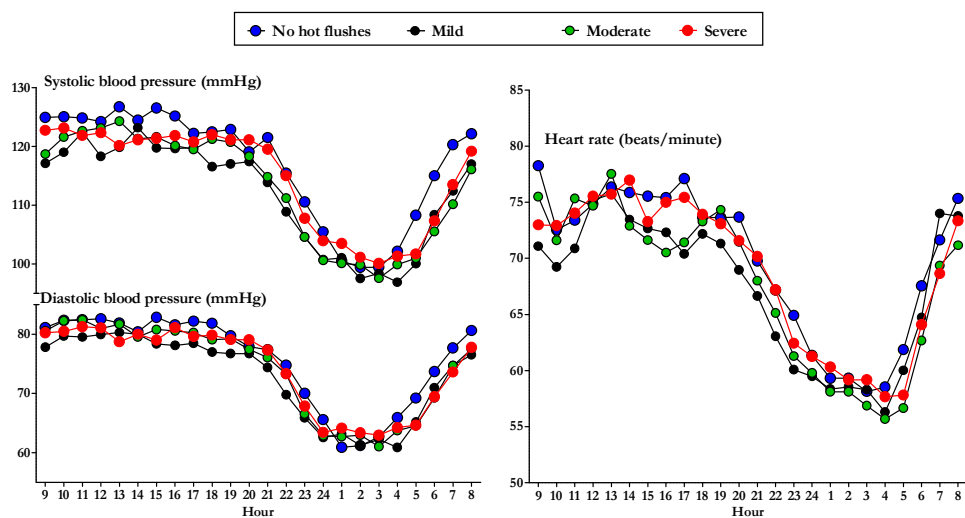


Figure 7. Mean contours of blood pressure and heart rate during 24 hours in women with no, mild moderate or severe hot flushes. Reprinted and slightly modified with permission from Informa UK Ltd.

However, severe night-time hot flushes were accompanied by transient increases in systolic ( $4.1 \pm 10.5$  mmHg,  $p=0.061$ ) and diastolic BP ( $3.1 \pm 6.8$  mmHg,  $p=0.032$ ), and heart rate ( $3.0 \pm 7.2$  beats/minute,  $p=0.043$ ) (Figure 8).

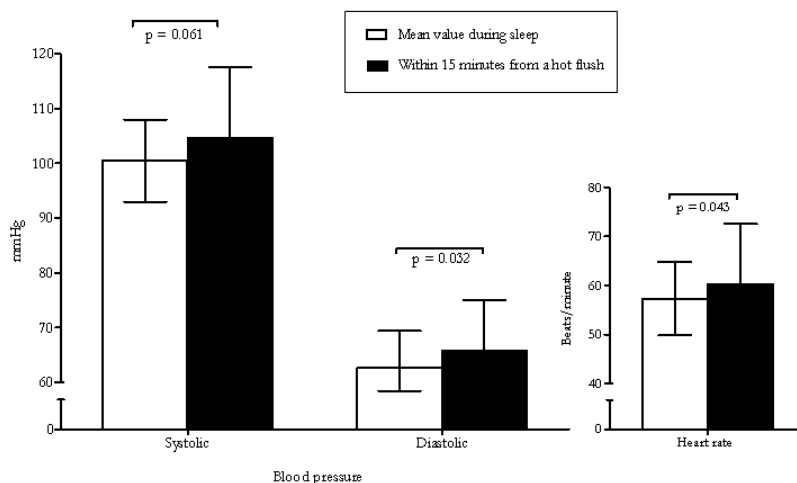


Figure 8. Impact of severe night-time hot flushes on ambulatory blood pressure and heart rate during 44 hot flush episodes in 26 women. Data are presented as mean $\pm$ SD. A  $p$ -value  $<0.05$  was considered statistically significant. Reprinted and slightly modified with permission from Informa UK Ltd.

# *Biochemical markers for cardiovascular disease (Study III)*

Hot flush status was not a determinant for the levels of lipids, lipoproteins, apolipoproteins or the ApoB/ApoA-1 ratio. Moreover, the levels of SHBG and hs-CRP were similar in women with no, mild, moderate or severe hot flushes. Estrogenic variables were also comparable between the study groups (Figure 9).

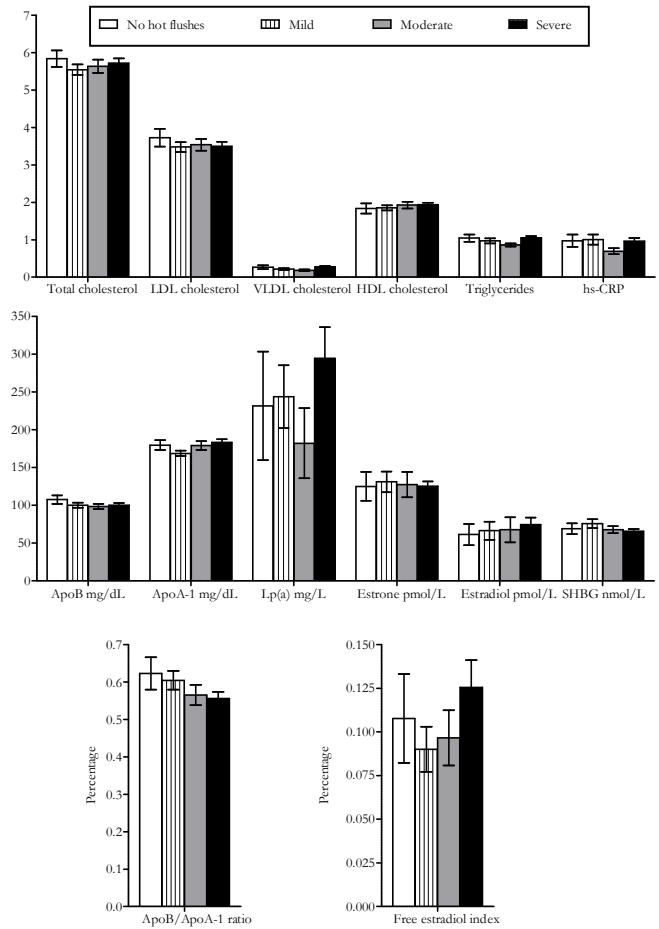


Figure 9. Levels of estrogens and biochemical markers of cardiovascular disease in postmenopausal women with hot flushes of different severities. Data presented as mean  $\pm$  SEM.

## Randomized trial on hormone therapy

All active treatments were equally effective in reducing vasomotor symptoms in women with intolerable hot flushes, whereas no effect was seen in women with tolerable hot flushes. All treatments irrespective of hot flush status caused significant rises in estrone and estradiol levels compared with placebo ( $p < 0.001$  for all). Transdermal estradiol caused an approximate 3-fold rise in estrone levels, which was smaller than the rises resulting from oral estradiol treatment with or without MPA (14-fold and 20-fold, respectively,  $p < 0.001$  for both). The rises in estradiol levels (approximately 3-4-fold) were comparable between all three active treatments.

## Responses of

### *Vascular function (Study IV)*

In women with tolerable hot flushes, oral estradiol caused significant ( $p < 0.05$ ) reductions in both time to peak ejection (~13%) and time to reflected waveform (~8%) after nitroglycerin as compared with the increases caused by transdermal estradiol (~11 and ~6%, respectively), oral estradiol complemented with MPA (~11% and ~7%, respectively), or placebo treatment (~8% and ~6%, respectively) (Figure 10). These changes were independent of time since menopause and changes in the levels of estrogens. In women with intolerable hot flushes both time to peak ejection and time to reflected waveform after nitroglycerin remained unaffected in all treatment groups. Overall, arterial stiffness (AIX) and endothelial function (EFI) were unaffected by the different treatment regimens or hot flush status.

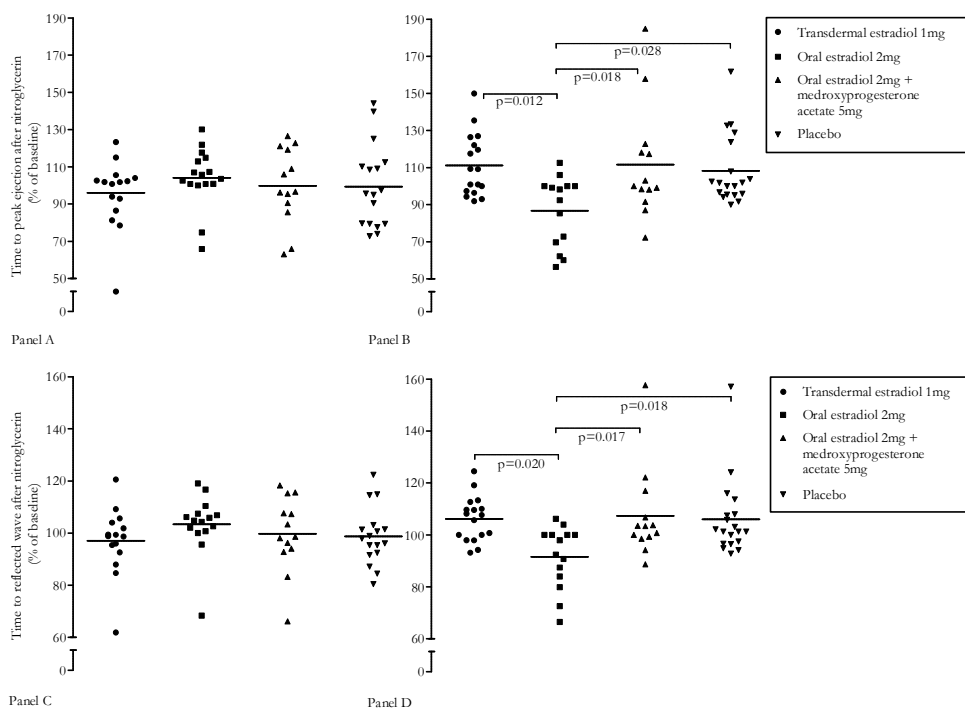


Figure 10. Vascular responses to different hormone therapy regimens and placebo. Time to peak ejection after nitroglycerin in women with intolerable (Panel A) and tolerable hot flushes (Panel B). Time to reflected waveform after nitroglycerin in women with intolerable (Panel C) and tolerable hot flushes (Panel D). Data presented as percentage of baseline with line at mean. A two-tailed  $p$ -value  $<0.05$  was considered statistically significant. Reprinted and slightly modified with permission from Wolters Kluwer Health.

## Ambulatory blood pressure (Study V)

Hot flush status was a significant determinant of the responses to HT of both 24-hour and daytime systolic and diastolic BPs. In women with tolerable hot flushes taking oral estradiol 24-hour systolic and diastolic BPs rose ( $3.7 \pm 1.2$  mmHg and  $1.8 \pm 0.8$  mmHg, respectively), whereas decreases ( $-1.2 \pm 1.2$  mmHg and  $-2.1 \pm 0.8$  mmHg, respectively) were detected in women with intolerable hot flushes ( $p=0.010$  and  $p=0.003$ , respectively) (Figure 11). Similarly, daytime systolic and diastolic BPs increased ( $3.0 \pm 1.3$  mmHg and  $1.8 \pm 0.9$  mmHg, respectively) in women with tolerable hot flushes receiving oral estradiol, but the same therapy led to falls ( $-1.9 \pm 1.3$  mmHg and  $-2.4 \pm 0.9$  mmHg, respectively) in women with intolerable hot flushes ( $p=0.017$  and  $p=0.003$ , respectively) (Figure 11). A tendency towards similar responses was observed for night-time systolic and diastolic BPs, but these did not reach statistical significance. Transdermal estradiol and oral estradiol complemented with MPA caused comparable responses in 24-hour, daytime and night-time BPs. Furthermore, in women with tolerable hot flushes the use of transdermal estradiol led to significant decreases in 24-h systolic and diastolic BPs compared with the increases caused by oral estradiol ( $p=0.001$  and  $p=0.041$ , respectively). Hot flush status was not a determinant of the responses in heart rate or nocturnal dipping to the different treatment regimens. The baseline levels were significant determinants of the changes in BPs and heart rate, but no dependence on time since menopause or changes in levels of estrone, estradiol, or the FEI was found.

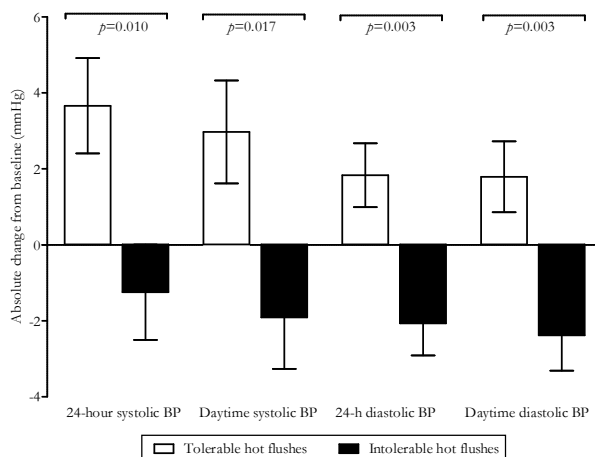


Figure 11. Effect of oral estradiol on 24-hour and daytime systolic and diastolic BPs in women with tolerable and intolerable hot flushes. Data presented as mean  $\pm$  SEM adjusted for the respective baseline levels. A  $p$ -value  $<0.05$  was considered statistically significant.

## Biochemical markers for cardiovascular disease (Study VI)

Hot flush status did not affect the responses of biochemical markers for CVD to various forms of HT. When all active treatments (estradiol administered either transdermally, or orally; alone or together with MPA) were assessed together, 7 out of 10 markers, i.e. total cholesterol, LDL, VLDL and HDL cholesterol, apoB, SHBG and hs-CRP, showed more beneficial changes during estradiol use in women with intolerable versus tolerable hot flushes, whereas the changes in triglycerides, Lp (a) and apoA-I were more favorable in women with tolerable hot flushes. Furthermore, in women with intolerable hot flushes treated with any form of estradiol, the rises in serum SHBG concentrations ( $\sim 73\%$ ,  $n=45$ , Spearman's rho  $-0.570$ ,  $p<0.001$ ) were related to falls in HFWWS, but no other statistically significant relations emerged (Figure 12).

On the whole, oral estradiol caused significant increases in HDL ( $\sim 13.5\%$ ), apoA-1 ( $\sim 10.5\%$ ), SHBG ( $\sim 116.7\%$ ), and hs-CRP ( $\sim 110.3\%$ ) as compared with placebo. Adding MPA to oral estradiol treatment significantly blunted the rises of both HDL ( $\sim 6.4\%$ ) and SHBG levels ( $\sim 50.3\%$ ), and caused a significant reduction ( $\sim 7.9\%$ ) in total cholesterol levels. Compared with placebo transdermal estradiol was effective only in decreasing levels of apoB ( $-7.8\%$ ). Analyses were controlled for the influence of time since menopause and changes in the levels of estrogens, and these were not determinants of the responses of biomarkers.

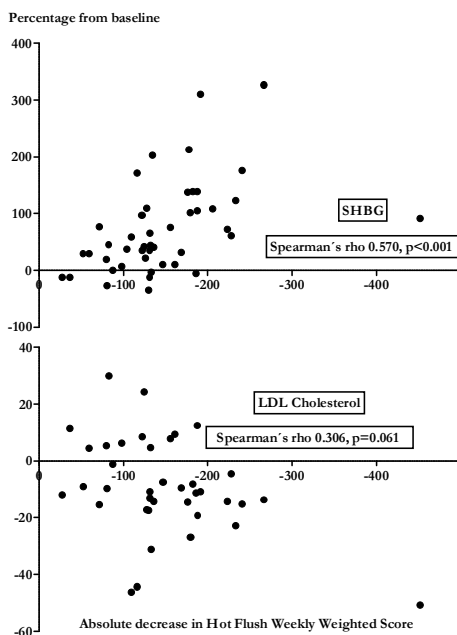


Figure 12.

Correlations between the decreases in the HFWWS and changes in SHBG and LDL cholesterol levels in women with intolerable hot flushes at baseline after receiving six months of any hormone therapy. A two-tailed  $p$ -value  $<0.05$  was considered statistically significant.

## DISCUSSION

Several experimental and human data imply that hypoestrogenism is associated with accelerated atherosclerosis (*Mikkola and Clarkson 2002*). This, at least in part, may explain the increase in the risk of CVD in postmenopausal women (*Shaw et al. 2009*), and also the fact data that postmenopausal HT has been accompanied with a 40–50% reduction in the incidence of CVD in observational studies (*Stampfer and Colditz 1991, Grady et al. 1992, Grodstein et al. 2000*). These findings, however, were not confirmed in randomized, placebo-controlled trials, which showed not only a lack of benefits, but rather cardiovascular harm in the early phase of HT use (*Hulley et al. 1998, Grady et al. 2002, Rossouw et al. 2002, Manson et al. 2003, Anderson et al. 2004*). Exploring the possible explanations for these divergent data was one primary goal of my research, and thus, I explored the role of postmenopausal hot flashes in connection with cardiovascular health.

After the initiation of my study, data have accumulated to show that a woman's age and the time since the last menstruation may significantly alter the cardiovascular effects of HT with potential harm accumulating in the more elderly women (*Grodstein et al. 2006, Hsia et al. 2006, Ouyang et al. 2006, Lobo 2007, Manson et al. 2007, Rossouw et al. 2007, Hernan et al. 2008, Vitale et al. 2008*). This may be related to the decrease of estrogen receptors in vascular walls with advancing age (*Kuhl 2005*). Moreover, it is possible that early atherosclerotic changes, which unavoidably develop in each individual, enhance the risk of vascular harm associated with HT. In other words, the effects of HT may be deleterious in diseased blood vessels even though they are beneficial in healthy blood vessels (*Mikkola and Clarkson 2002*). Therefore, there may be a window (age or time since menopause) for the opportunity to prevent CVD by HT; early initiation of HT may be protective, whereas late initiation may be detrimental (*Mikkola and Clarkson 2002, Mikkola and Ylikorkala 2005, Mikkola and Clarkson 2006*). This theory is also supported by later analyses of WHI data (*Hsia et al. 2006, Manson et al. 2007, Rossouw et al. 2007*), although not all experts seem to share this view (*Prentice et al. 2009*). To conclude, the current understanding is that the use of HT is protective against CVD in healthy women between 50–59 years, but not protective and even harmful if initiated after 60 years of age (*Koledova and Khalil 2007, van de Weijer 2008, Lobo 2009*).



The presence of vasomotor hot flushes has emerged as one possible contributing factor for the divergent results (*Mikkola and Ylikorkala 2005, van der Schoon and Grobbee 2005, Mendelsohn and Karas 2007, Allison and Manson 2009, Andrikoula et al. 2009, Pinkerton and Stovall 2009*). In clinical practice, women with troublesome hot flushes seek HT treatment, and thus, such women were included into HT users in observational studies (*Mikkola and Ylikorkala 2005*). In contrast, hot flushes were mild or absent in the majority of women taking part in the WHI trial, for example, in part as a result of the fact that women with severe vasomotor symptoms would not comply with possibly being randomized to a placebo group (*The Women's Health Initiative Study Group 1998*).

Differences in the cardiovascular effects of HT have also in part been attributed to varying contents of hormone regimens, i.e. the relative high dose of CEE 0.625mg and the use of continuous MPA, which were the compounds used both in the HERS and the WHI trials (*Grodstein et al. 2003, van der Schoon and Grobbee 2005, Kuhl and Stevenson 2006*). The impact of MPA on the vascular effects of HT has been particularly widely debated (*Clarkson and Appert 2003, Koh and Sakuma 2004, Kuhl and Stevenson 2006*). This progestagen has antiestrogenic, androgenic and glucocorticoid properties which may counteract the beneficial effects of estrogen (*Kuhl 2005, Sitruk-Ware 2007b*). Thus, it is possible that the vascular responses to estrogen with and without MPA differ (*Adams et al. 1997, Sitruk-Ware 2000, Kavano et al. 2001*), although this is not supported by the results of all clinical studies (*Clarkson and Appert 2003, Yeboah et al. 2007*). It is also known that adding MPA to the regimen may prevent estrogen-induced rises in biomarkers (*Godsland 2001, Shulman 2002*). For instance, oral estrogen alone or in combination with low-dose MPA (2.5 mg/day) increases the levels of C-reactive protein, but not if oral estrogen is accompanied by a higher dose (5.0 mg/day) of MPA (*Wakatsuki et al. 2002*). Furthermore, it is known that the addition of MPA to estradiol for two weeks does not affect the responses of SHBG or hs-CRP to oral estradiol alone (*Ropponen et al. 2005a, Ropponen et al. 2005b*). Since the data regarding MPA are diverse, it was of special interest to investigate the effects of this progestagen in my study. As regards estrogen, CEE contains a variable mixture of at least ten estrogen sulfates, only three of which are produced in humans, and may therefore yield different clinical effects (*Kuhl 2005*). Thus, to be able to adequately determine the specific effects of estrogen, estradiol was chosen as the estrogen component for my study.

To assess the impact of hot flushes, the study population was carefully chosen to represent healthy, postmenopausal women with no known cardiovascular risk factors. Additional strengths of my study include the use of prospective recording and validated scoring of hot flushes, which enables accurate assessment of vasomotor symptoms (*Sloan et al. 2001*). Vascular health was evaluated using a multimarker approach, which has been suggested to

be the most representative tool to assess the overall cardiovascular health (*Ikonomidis et al. 2008*). The following points should be acknowledged. Firstly, PWA provides assessment of both structural and functional properties of arteries, especially if combined with endothelial testing (*Davies and Struthers 2003, Hirata et al. 2006, Kullo and Malik 2007, Wittrock et al. 2008*). Secondly, BP was measured over 24 hours with ambulatory BP registration, which is the gold standard of BP assessment in clinical research (*Pickering et al. 2006*). Thirdly, the atherosclerotic and inflammatory burdens of the vascular bed were evaluated with established biochemical markers for CVD (*Ridker et al. 2000, Knopp 2002, Carmena et al. 2004, Chan and Watts 2006*).

After the baseline assessments the study subjects were randomized to oral and transdermal estradiol at equipotent doses (*Kubl 2005, Panay 2009*), which enabled comparison of the impact of different routes of estradiol administration. It was already known before the design of the present study that the cardiovascular effects of orally and transdermally administered HT may differ (*Godsland 2001*), and this evidence became stronger during the study (*Khalil 2005, Modena et al. 2005, Clarkson and Karas 2007, Hemelaar et al. 2008, L'Hermite et al. 2008*).

As limitations of the study I acknowledge that the study population consisted of lean, white women and thus my results are not generalizable to obese women or women belonging to other ethnic groups. Moreover, I studied only normotensive women, and the effect of HT in hypertensive women may be different. This reservation is significant; many postmenopausal women show slightly elevated BP, perhaps as a consequence of hot flashes (*Vongpatanasin 2009*) and, in theory, the effect of HT might be most conspicuous in women with hot flashes and mildly elevated BP. Furthermore, my study subjects had entered menopause within the preceding 0.5 to 3 years, so my data may not hold for older women with longer postmenopausal age accumulation. In addition, HT was given only for six months and a longer treatment period might have yielded additional differences. However, 2- to 6-month HT treatments have commonly been used in HT studies (*Mueck and Seeger 2004, Teede 2007*). In addition, estradiol was given at conventional doses, and modern low doses may show different effects (*Langer 2009*). Moreover, my data may not be applicable for other estrogen/progestagen combinations. Finally, I studied a limited number of women and therefore, it is possible that some changes remained undetected due to the small sample sizes. However, the power of the study was calculated to detect the impact of hot flashes on the outcomes of PWA.

## Vascular function

Menopause may increase arterial stiffness, perhaps as a consequence of hypoestrogenism (*Takahashi et al. 2005, Zaydun et al. 2006*). Older data have shown increased venous blood flow in the upper extremities of flushing women (*Ginsburg et al. 1981, Ginsburg et al. 1989*). My data demonstrate that increasing severity of hot flushes is accompanied by an arterial vasodilatory feature, as evidenced by prolongations of the times to peak ejection and reflected waveform after the nitroglycerin challenge. This may indicate a favorable vascular feature in women with hot flushes of different severities. The presence of hot flushes was not associated with any changes in arterial stiffness or endothelial function (Study I).

During the course of my study some data have emerged that suggest lower FMD in women with hot flushes (*Thurston et al. 2008a*). There are marked differences between my study and that of Thurston and coworkers. First, the women studied by Thurston and colleagues were obese (mean BMI 29 kg/m<sup>2</sup>), whereas participants in my study were lean (mean BMI 23 kg/m<sup>2</sup>) (Study I). Second, approximately one third of the participants in Thurston's study were African-American women who are known to have in general a less favorable cardiovascular risk profile (*Winkleby et al. 1998*), whereas I studied Caucasian women. Third, the population in the study by Thurston et al. included also women using HT, and thus they cannot be compared with the HT-naïve women in my study. Finally, and perhaps most importantly, hot flushes were retrospectively recalled and the estradiol levels were almost twice as high in flushing women as in women without hot flushes (*Thurston et al. 2008a*), whereas hot flushes in my study were prospectively recorded and the study groups did not differ in estradiol levels (Studies I, II and III).

Besides studying the impact of hot flushes on vascular variables in a cross-sectional setting, the present study evaluated the impact of hot flushes on outcomes of HT in a prospective, randomized trial. Previous data on the impact of HT on vascular function are inconclusive due to the diversity in study methodologies (*Ganz 2002, Koh 2002, Teede 2007, Bechlioulis et al. 2009*), but in some studies HT has been shown to reduce arterial and aortic stiffness and improve endothelial function (*Hayward et al. 1997, Miura et al. 2003, Szejnszajd et al. 2006, Vitale et al. 2008*). In my study the use of oral estradiol was associated with significant reductions in times to both peak ejection and reflected waveform after nitroglycerin, but only in women with tolerable hot flushes (Study IV). This can be seen as a vasoconstrictive response to oral estradiol in these women. Interestingly, in women with intolerable hot flushes, the responses to transdermal estradiol or oral estradiol with or without concomitant MPA were neutral in terms of vasoconstriction or vasodilation. Arterial stiffness and endothelial function remained unaffected in all the study groups (Study IV).

Thus, taken as a whole, the route of estrogen appears to be a critical factor; the transdermal route compares favorably with the oral one, at least in women without troublesome hot flushes.

## **Ambulatory blood pressure**

Elevations in sympathetic nervous activity due to hypoestrogenism are found in women with vasomotor symptoms (*Freedman 2005, Deecher and Dorries 2007, Sturdee 2008*), and this could result in elevated BP (*Coylewright et al. 2008, Vongpatanasin 2009*). Prior to my study some data implied that hot flushes were associated with an increase in either office (*Beljic et al. 1999*) or ambulatory BP (*Brown et al. 2001, James et al. 2004*). During the course of my study additional data from cross-sectional studies have shown an association between hot flushes and increases in office (*Gast et al. 2008, Thurston et al. 2008a, Gallicchio et al. 2009*) or ambulatory BP (*Gerber et al. 2007*). However, none of these studies utilized the recommended procedure of prospective hot flush recording, but rather a retrospective recall, which certainly is inaccurate (*Pinkerton and Stovall 2009*). Furthermore, these studies included smokers (*Brown et al. 2001, James et al. 2004, Gerber et al. 2007, Gast et al. 2008, Thurston et al. 2008a, Gallicchio et al. 2009*) and hypertensive women (*Gerber et al. 2007, Gast et al. 2008, Gallicchio et al. 2009*) and thus these data are not generalizable to a healthy and HT-naïve population as I studied; my study population represents the majority of women considering the use of HT in routine clinical practice. Hot flushes have been shown to associate with simultaneous increases (*Freedman and Dinsay 2000, Low et al. 2008*) or decreases in BP (*Nelesen et al. 2004*). My data show that severe hot flushes are associated with increases in night-time diastolic BP and heart rate. However, these increases were so short that on the whole, the overall effect of hot flushes on diurnal BP was negligible (Study II). However, heart rate variability may be a more sensitive marker of cardiovascular function than sole heart rate or BP, and this variability shows an interesting association with severe hot flushes (*Hoikkala et al. 2010*).

Hormone therapy modifies a number of vasoregulators, and therefore, HT may affect BP (*Luotola 1983, Khalil 2005, Koledova and Khalil 2007*). The progestagen component in HT may also modify BP. For instance, drospirenone, a recently introduced progestagen with antimineralocorticoid activity, present both in oral contraceptive pills and menopausal EPT regimens (*Anttila et al. 2009*), may lower BP (*Nath and Sitruk-Ware 2009, Preston 2009*). Decreases in BP have been associated with transdermal estradiol use (*Cacciatore et al. 2001, Mueck and Seeger 2004, L'Hermite et al. 2008*). On the other hand, oral estradiol may increase BP, perhaps by stimulating the production of renin substrate in the liver; this results in the

release of angiotensin-II, one of the most potent hypertensive agents (*Miller and Duckles 2008*). My data show that the presence of hot flushes affects BP responses according to the type of HT (Study V). In women with tolerable hot flushes oral estradiol was accompanied by increases in both 24-h and daytime BPs, whereas no such effect was seen in women with intolerable hot flushes. This is clinically significant; the former group is usually not a candidate for hormone use, whereas the latter women typically are. Deterioration in the diurnal variation of BP may occur in menopause and HT may improve this by increasing nocturnal BP dipping (*Butkevich et al. 2000, Wong et al. 2005*). My data, however, did not show such an effect of HT, perhaps because all participants already had a normal dipping pattern at baseline. The situation might have been different if older women or women with mildly elevated BP were to have been included.

The message for the clinician reads; if a symptomless woman needs estrogen for a reason other than for the control of hot flushes, favor transdermal administration, because this may provide some vascular benefits.

## **Vascular biochemical markers**

When this study was planned, only a reduced antioxidant status was known to be related to hot flushes (*Leal et al. 2000*). During my study, data were published linking hot flushes to risk factors of CVD, such as increased abdominal adiposity (*Thurston et al. 2008b*) and elevated cholesterol levels (*Gast et al. 2008, Thurston et al. 2008a*). However, the majority of the studies were cross-sectional studies that included pre-, peri- and postmenopausal women, and women on HT or tobacco users, and women with hypertension and therefore, the results may not truly reflect the causality between hot flushes and various markers for CVD. My data, concerning in healthy, recently postmenopausal women, show no association between baseline hot flush status and biochemical markers for CVD (Study III).

There are no previous data on the impact of baseline hot flush status on the responses of biochemical CVD surrogate markers to HT. My data demonstrate that hot flush status at baseline did not significantly affect the responses of various biochemical markers for CVD to HT, although the majority of markers (70%) showed a tendency towards more beneficial vascular responses in women with intolerable hot flushes (Study VI). As regards the overall effect of various forms of HT, my data are in line with most previous data; oral estradiol is accompanied by more pronounced beneficial changes in lipid and lipoprotein levels than transdermal estradiol with the exception of triglycerides, which do not substantially increase

with transdermal treatment (*Tikkanen 1996a, Tikkanen 1996b, Godsland 2001, Kubl 2005, Hemelaar et al. 2008*). In my study the most pronounced decreases in the levels of total cholesterol were detected in connection with transdermal and oral estradiol, an outcome, however, which may be a result of the small sample size. The addition of MPA to oral estradiol may prevent some of the estradiol-induced changes in biomarkers due to its antiestrogenic properties (*Sitruk-Ware 2004, Hermismeyer et al. 2008*); such an effect of MPA was also seen in my study and most conspicuously in HDL, apoA-I and SHBG (Study VI).

In recent years several re-analyses of HT trials have been made to evaluate the effect of hot flushes on the outcomes of HT. Re-analysis of the WHI data showed that the presence of moderate hot flushes at baseline was associated with a non-significantly lower risk of CVD in women aged 50–59 years (HR 0.86, 95% CI 0.44–1.65) (*Rossouw et al. 2007*). Interestingly, data regarding the effects of HT in older women with hot flushes are more divergent. In the WHI study women aged 70–79 years with moderate to severe hot flushes at the initiation of HT, had an increased risk of CHD associated with HT use (HR 5.08, 95% CI 2.08–12.40) (*Rossouw et al. 2007*). Furthermore, re-analyses of the HERS (*Huang et al. 2009*) and Rancho Bernardo (*Svartberg et al. 2009*) studies showed that in women over 65 years of age, hot flushes at baseline seem to be associated either with an increased risk of CHD during the first year of HT use (HERS; HR 9.01, 95% CI 1.15–70.35) or a lower all-cause mortality rate (Rancho Bernardo; HR 0.72, 95% CI 0.55–0.94), respectively. This diversity in results is interesting and warrants further research, since these trials were initially not designed to assess the impact of hot flushes, and the analyses were made retrospectively, when data on the influence of hot flushes had accumulated.

Future directions for research include a need to assess the impact of hot flushes on glucose-insulin balance. Large, prospective studies are needed to confirm the impact of hot flushes on outcomes of HT, and in future trials hot flush status should be considered a potential confounding factor. Furthermore, research should be directed to women with established cardiovascular risk factors at baseline, such as mild hypertension, smoking or hypercholesterolemia. Research should also be focused on older women in their 60s and 70s who still have hot flushes.

In conclusion, HT is the most effective treatment for hot flushes. Healthy, recently postmenopausal women with moderate to severe hot flushes should be given the opportunity to use estrogen alone (if hysterectomized) or in combination with an appropriate progestagen (if with intact uterus) to alleviate hot flushes and other symptoms. Possible contraindications for HT should be noted and cardiovascular risk factors should be evaluated and, if detected, treated accordingly; this particularly concerns elevated BP (*Collins et al. 2007, Utian et al. 2008, International Menopause Society Consensus Statement 2009*). My data show that the use of oral estradiol may present a risk of vasoconstriction and an increase of BP, but only in women with tolerable hot flushes. Luckily, in clinical practice today such women are seldom prescribed HT. However, if HT is needed for women with no or only mild hot flushes for other indications such as bone protection, transdermal HT should be favored.

## SUMMARY

The present studies conducted on healthy, recently postmenopausal women allow the following conclusions:

I                      Vasodilatory reactivity in women with severe hot flushes is greater than that in women with no, mild, or moderate hot flushes. Thus, vasomotor hot flushes could serve as marker of good vascular function.

II                     Hot flushes of different degrees of severity are not associated with BP or heart rate except at night, when severe hot flush episodes are followed by transient rises in BP and heart rate.

III                    Hot flushes of different degrees of severity are not associated with variation in the circulating concentrations of lipids, lipoproteins, SHBG or hs-CRP.

IV                    The use of oral estradiol alone was accompanied by a vasoconstrictive response in women with tolerable hot flushes, whereas no such effect was detected in women with intolerable hot flushes or with the other treatment regimens.

V                      Women with tolerable hot flushes responded to oral estradiol with elevations in both 24-hour and daytime systolic and diastolic BP. In contrast, use of transdermal estradiol resulted in decreases in 24-h and daytime BPs. In women with intolerable hot flushes decreases in BP were seen in connection with all treatment regimens.

VI                    The responses of circulating concentrations of lipids, lipoproteins, SHBG and hs-CRP to oral and transdermal estradiol, the former with or without MPA, were comparable in women with tolerable and intolerable hot flushes. However, women with intolerable hot flushes responded with beneficial changes in 70% of the markers.

The data demonstrate that hot flushes may affect various aspects of vascular function differently. Furthermore, they may clarify in part the conflict between the results of observational and randomized hormone studies. If HT is used for indications other than the control of hot flushes, a transdermal route of estrogen use should be favored.



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Helsinki, March 2010

A handwritten signature in black ink, appearing to read 'Pauliina', with a long horizontal stroke extending to the right.

Pauliina Tuomikoski

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